CHRONIC TOXICITY OF LOW DISSOLVED OXYGEN CONCENTRATIONS, ELEVATED pH, AND ELEVATED AMMONIA CONCENTRATIONS TO LOST RIVER SUCKERS (*DELTISTES LUXATUS*), AND SWIMMING PERFORMANCE OF LOST RIVER SUCKERS AT VARIOUS TEMPERATURES

Research Report Submitted to:

Mr. Mark Buettner
U.S. Department of the Interior
Bureau of Reclamation
Klamath Basin Area Office
6600 Washburn Way
Klamath Falls, OR 97603

Submitted by:

Joseph S. Meyer
Hilary M. Lease
Harold L. Bergman
Department of Zoology and Physiology
University of Wyoming
Laramie, WY 82071-3166

14 January 2000

EXECUTIVE SUMMARY

Two species of sucker (the shortnose sucker, *Chasmistes brevirostris*; and the Lost River sucker, *Deltistes luxatus*) inhabit Upper Klamath Lake, a eutrophic water body in southern Oregon. Extremes of water quality that regularly occur in the lake include elevated pH and ammonia concentrations during cyanobacterial blooms, and low dissolved oxygen (DO) concentrations during subsequent "crashes" of the cyanobacterial populations. Because both sucker species are federally listed as endangered, the effects of water quality on their survival, recruitment and general health is a major concern. In addition to the chemical stressors in Upper Klamath Lake, diversions from the lake and tributary streams can kill suckers through entrainment. Thus, the ability of these fish to swim away from a current can be crucial to the survival of individuals and to the viability of the population in the lake.

To provide a broader scientific basis for managers to address concerns about water quality, we conducted five chronic toxicity tests (14- or 30-d duration) with larval or juvenile Lost River suckers exposed to the following water-quality conditions:

- Late juveniles exposed to low DO concentrations (1-7 mg/L, with access to the water surface) for 14 days.
- Late juveniles exposed to low DO concentrations (1-7 mg/L, without access to the water surface) for 14 days.
- Larvae exposed to elevated pH (8-10) for 30 days.
- Larvae exposed to elevated ammonia concentrations (0-1.2 mg NH_3-N/L) at pH 9.5 for 30 days.
- Early juveniles exposed to sublethal ammonia concentrations (0-0.3 mg NH_3-N/L) at pH 9.5 for 14 days, followed by exposure to sublethal DO concentrations (2-7 mg/L) for 14 days.

Additionally, to address concerns about entraining currents, we conducted swimming performance tests over a range of water temperatures (7-25 C) with early-juvenile and late-juvenile Lost River suckers.

Mortality threshold ranges determined in the toxicity tests were between ~1.5 and 2.0 mg/L in the two DO tests, >10.0 in the pH test (highest pH tested), and between 0.37 and 0.69 mg NH_3-N/L in the ammonia test. Contrary to the common expectation for fish chronically exposed to toxicants, Lost River suckers generally did not display sublethal responses to low DO concentrations, elevated pH, or elevated ammonia concentrations, based on the three traditional chronic-toxicity endpoints we used (growth, whole-body ion content, and swimming performance). In the two 14-day exposures to low DO concentrations and in the 30-day exposure to elevated ammonia concentrations, the traditional sublethal endpoints were no more sensitive than survival was; only in the 30-day exposure to elevated pH was a sublethal endpoint (whole-body ion content) more sensitive than survival. In the 14-d sublethal ammonia:14-d sublethal DO test, mortality did not decrease significantly and no sublethal effects were observed. Although large increments between exposure concentrations can cause an apparent absence of sublethal responses in a chronic toxicity test, we had relatively narrow increments (0.5 mg DO/L, 0.5 pH units, and 0.32 mg NH_3-N/L) between the effects and no-effects exposures in these tests. Therefore, it appears that, within the resolution of the traditional chronic-toxicity endpoints we used, a Lost River sucker essentially had to be dying before an adverse *functional* effect of the toxicant could be identified.

On the other hand, gill histopathology (a less traditional index of sublethal effects) was

sometimes more sensitive than the three traditional chronic endpoints. In the ammonia test, statistically significant structural changes occurred in gills of larvae exposed continuously to unionized ammonia concentrations 3.5 times lower than the lowest concentration at which significant mortality and growth effects occurred. Changes in gill structure that we quantified included significantly increased oxygen diffusion distance and increased thickness of secondary lamellae -- the primary site for respiratory and ionoregulatory exchange in a fish. Additionally, we observed qualitative structural changes, including increased numbers of chloride and mucous cells, the appearance of mitotic figures, and infiltration of white blood cells into the lymphatic space. However, we did not detect significant structural changes in gills of fish exposed to the highest sublethal pH (10.0, the highest level tested) in the pH test. Because of technical difficulties with tissue embedment, we did not analyze gills in the two 14-d DO tests or in the 14-d sublethal ammonia:14-d sublethal DO test.

From a management perspective, the exposure increments in our toxicity tests probably were narrow enough to help establish targets for remediation of water quality in Upper Klamath Lake. In general, Lost River suckers did not display major *functional* physiological impairment at concentrations in which no significant mortalities occurred (i.e., $\geq$2.0 mg DO/L, $\leq$ pH 10.0, and $\leq$ 0.37 mg NH_3-N/L). However, structural damage to gill tissue can occur at sublethal exposure conditions and might be a useful sublethal index of acceptable water quality for in-lake monitoring -- whether or not the tissue damage is severe enough to impair the functioning of the fish.

Body size and water temperature affect the critical swimming speed (CSS) of juvenile Lost River suckers. The following multiple-regression equations can be used to predict absolute (CSS) or body-length-normalized (CSS_{BL}) critical swimming speeds:

$$CSS = 0.131 \cdot L + 0.574 \cdot T + 7.732 \quad (R^2 = 0.464,\ P < 0.001)$$

and

$$CSS_{BL} = -0.0441 \cdot L + 0.0916 \cdot T + 5.448 \quad (R^2 = 0.546,\ P < 0.001)$$

where CSS is in cm/s, CSS_{BL} is in body lengths/s, L = body length (mm), and T = water temperature (C). Standard errors for the three constants in the first equation are 0.020, 0.046 and 1.420, respectively; whereas standard errors for the three constants in the second equation are 0.003, 0.008 and 0.239, respectively. These equations should only be used within the ranges of body length and water temperature that we tested (~40-100 mm and 7-25 C). Size- and temperature-dependent differences in swimming speed could be an important management consideration if water is pumped from Upper Klamath Lake at different times of the year when fish size and water temperature differ.

CONTENTS

CONTENTS (continued)

Page

TABLES

TABLES (continued)

FIGURES

ACKNOWLEDGEMENTS

This research was funded by the Bureau of Reclamation (U.S. Department of the Interior) through an interagency agreement with the Wyoming Cooperative Fisheries and Wildlife Research Unit of the U.S. Geological Survey (USGS), located in the Department of Zoology at the University of Wyoming. Dr. James Hansen, Larry DeBrey, Preston Kash and Connie Boese helped to conduct the toxicity and swimming-performance tests and performed chemical analyses at the University of Wyoming; and Joe Bobbitt assisted with the design and construction of the experimental system. Larry Dunsmoor (Klamath Tribes Native Fish Hatchery) supplied the Lost River suckers and advised us on culturing techniques. Mark Buettner (Bureau of Reclamation), Larry Dunsmoor, Jake Kann (Klamath Tribes) and Dave Perkins (USGS Biological Resources Division) provided input to the study design. The Wyoming Department of Game and Fish granted permission to conduct tests with Lost River suckers at Red Buttes Environmental Biology Laboratory.

INTRODUCTION

Two species of sucker (the shortnose sucker, *Chasmistes brevirostris*; and the Lost River sucker, *Deltistes luxatus*) inhabit Upper Klamath Lake, a eutrophic water body in southern Oregon. Although the lake historically has been eutrophic, numerous land- and water-use alterations in the drainage have increased the nutrient inflow and made the lake hypereutrophic; additionally, water removal for irrigation, hydroelectric generation, wildlife refuges, and instream flows for fish have lowered the water levels below historic levels (Kann and Walker 1999). Water-quality deterioration and habitat loss associated with those changes have contributed to drastic declines in the sucker populations since at least the mid-1960s (USFWS 1993). In 1988, both species were federally listed as endangered (USFWS 1988). Massive sucker mortalities that continue to occur during most summers in Upper Klamath Lake are thought to be caused by stressful conditions associated with the poor water quality (USFWS 1993; Perkins et al., manuscript).

Water quality in Upper Klamath Lake varies considerably, cycling on a daily and a seasonal basis. In part, this water-quality cycling is due to seasonal cycles in phytoplankton activity, especially by the cyanobacterium *Aphanizomenon flos-aquae* (Saiki et al. 1999). For example, limnological data presented in Kann (1998), Kann and Smith (1999), Perkins et al. (manuscript) and Kann (written communication, Dr. Jacob Kann, Aquatic Ecosystem Sciences, Ashland, Oregon, 20 October 1997) demonstrate that pH can exceed 10 on some summer days, with common pH maxima between 9 and 10 and 24-h fluctuations of up to 2 pH units; dissolved oxygen (DO) can be depleted to 0 mg/L on some nights but can reach daytime highs approaching 15 mg/L a day or two later, with 24-h fluctuations of >10 mg/L; and unionized ammonia concentrations sometimes exceed 1.0 mg NH_3-N/L (although no data are available for daily cycling of ammonia concentrations). Usually, these extreme conditions occur when daytime temperatures are ~25-30^{o}C.

Survival, recruitment and general health of the suckers in such extreme water quality is a major concern. Short-term (e.g., 96-h) toxicity tests have been conducted with Lost River suckers and shortnose suckers exposed to low dissolved oxygen concentrations, high pH, and high ammonia concentrations in the laboratory (e.g., Falter and Cech 1991, Monda and Saiki 1993, Monda and Saiki 1994, Bellerud and Saiki 1995, Saiki et al. 1999); and caged juvenile Lost River suckers were exposed in situ for up to 96 hours to a variety of water-quality conditions occurring in Upper Klamath Lake during summer 1995 (Martin 1997, Martin and Saiki 1999). However, no toxicity data are available for long-term exposures to such conditions. Thus, we conducted a series of chronic toxicity tests in which Lost River suckers were exposed to low dissolved oxygen concentrations, elevated pH, and high ammonia concentrations.

In addition to the chemical stressors in Upper Klamath Lake, diversions from the lake and tributary streams can kill suckers through entrainment (USFWS 1993:18). Thus, the ability of these fish to swim away from an entraining current can be crucial to the survival of individuals and to the viability of the population in the lake. Delonay and Little (1997) conducted a study of swimming performance of juvenile Lost River suckers and shortnose suckers at 17 C. However, because (1) temperature can affect fish metabolism and swimming speed, and (2) water temperature varies during the period when water is withdrawn from Upper Klamath Lake, we conducted a follow-up study of the swimming performance of juvenile Lost River suckers across a range of water temperatures.

METHODS

Study Design

We conducted five chronic toxicity tests (14- or 30-d duration) with larval or juvenile Lost River suckers exposed to the following water-quality conditions:

- Late juveniles exposed to low DO concentrations (1-7 mg/L, with access to the water surface) for 14 days.
- Late juveniles exposed to low DO concentrations (1-7 mg/L, without access to the water surface) for 14 days.
- Larvae exposed to elevated pH (8-10) for 30 days.
- Larvae exposed to elevated ammonia concentrations (0-1.2 mg NH_3-N/L) at pH 9.5 for 30 days.
- Early juveniles exposed to sublethal ammonia concentrations (0-0.3 mg NH_3-N/L) at pH 9.5 for 14 days, followed by exposure to sublethal DO concentrations (2-7 mg/L) for 14 days.

Additionally, we conducted swimming performance tests over a range of water temperatures (7-25 C). All of these tests were conducted at the University of Wyoming's Red Buttes Environmental Biology Laboratory (RBEBL), located 16 km south of Laramie, Wyoming. The suckers were shipped from the Klamath Tribes Native Fish Hatchery in Chiloquin, Oregon to RBEBL by Mr. Larry Dunsmoor.

Toxicity Tests

Exposure waters in the five toxicity tests were prepared from a base water that was a mixture of ~80% hard, alkaline well water and ~20% reverse-osmosis-treated and deionized well water. Nominal water-quality characteristics of the base water: pH - 7.5, hardness and alkalinity - 50 mg/L as $CaCO_3$ each, and conductivity - 125 µS/cm. Water temperature was maintained at 22 C in all of the toxicity tests, and the daily photoperiod was 14 h light and 10 h darkness. Fish were fed several times daily during each toxicity test (30% *Spirulina* + 70% Cyclop-eeze (Argent) in the larval and early-juvenile tests; brine shrimp in the late-juvenile tests), and the excess food and feces were siphoned from the aquaria 1 hour after feeding. All treatments and controls were replicated with four exposure aquaria each.

Toxicity Test #1 -- late juveniles exposed to low DO concentrations (with access to the water surface) for 14 days

We exposed late-juvenile Lost River suckers continuously to nominal DO concentrations of 1, 2, 3, 4, 5 and 7 (control) mg/L for 14 days, from 26 January to 9 February 1998. This exposure duration was chosen because inspection of limnological data (written communication, Dr. Jacob Kann, Aquatic Ecosystem Sciences, Ashland, Oregon, 20 October 1997) indicated that periods of low DO concentrations in surface waters of Upper Klamath Lake usually do not last longer than several weeks. The nominal DO concentration of 7 mg/L in the controls is ~100% saturation at 22 C and 2,200 m above sea level (the elevation of RBEBL).

At the beginning of the test, we placed 15 late juveniles in each of 24 glass aquaria (4 replicates per each of the six DO concentrations). The aquaria were 25-cm wide x 30-cm high x

50-cm long and contained 15 L of water. Water flowed continuously to each of the aquaria at a rate of 300 ml/min from a proportional diluter in which deoxygenated water and DO-saturated water were mixed to produce the desired DO concentrations. Water was deoxygenated by passing it through a vacuum degassing system (Fig. 1) constructed at UW.

The size of the fish at the beginning of the test was (average $\pm$ s.d.): length = 70.5 $\pm$ 5.59 mm, range = 58-82 mm, n = 50; weight = 3.63 $\pm$ 0.783 g, range = 2.22-4.96 g, n = 50.

In this first DO test, the fish were allowed access to the water surface.

Toxicity Test #2 -- late juveniles exposed to low DO concentrations (without access to the water surface) for 14 days

Similar to Toxicity Test #1, we exposed late-juvenile Lost River suckers continuously to nominal DO concentrations of 1, 2, 3, 4, 5 and 7 (control) mg/L for 14 days, from 2 to 15 March 1998. However, because fish in the lower DO concentrations in Toxicity Test #1 spent considerable amounts of time gulping at the water surface, we prevented the fish in Toxicity Test #2 from having access to the water surface by immersing a flat piece of plastic mesh about 2.5 cm deep in the water. The plastic mesh was held rigid by a plastic frame that was the same size as the horizontal cross-section of the aquarium. All other details of Toxicity Test #2 were the same as in Toxicity Test #1 (see above).

The size of the fish at the beginning of the test was (average $\pm$ s.d.): length = 72.0 $\pm$ 5.74 mm, range = 60-94 mm, n = 50; weight = 3.65 $\pm$ 0.957 g, range = 1.71-7.27 g, n = 50.

Toxicity Test #3 -- larvae exposed to elevated pH for 30 days

We exposed larval Lost River suckers continuously to nominal pH's of 8.0 (control), 9.0, 9.5 and 10.0 for 30 days, from 30 April to 29 May 1998. This exposure duration was chosen because inspection of limnological data (written communication, Dr. Jacob Kann, Aquatic Ecosystem Sciences, Ashland, Oregon, 20 October 1997) indicated that periods of elevated pH ranging from 9 to 10 (and sometimes higher) in surface waters of Upper Klamath Lake can last one month or longer. The nominal pH 8.0 of the controls is typical of Upper Klamath Lake when cyanobacterial blooms do not dominate lake-water chemistry. Rapid and massive precipitation of $CaCO_3$ in our diluter system prevented us from testing pH 10.5; and even at pH 10.0, we had to rinse the splitter boxes and tubing every few days to prevent accumulation of $CaCO_3$ that would have altered rates of water delivery to the exposure aquaria.

At the beginning of the test, we placed 25 larvae in each of 16 glass aquaria (4 replicates per each of the four pH's). The aquaria were 6.5-cm wide x 6.5-cm high x 18.5-cm long and contained 0.75 L of water. Water flowed continuously to each aquarium from a head tank at a rate of 50 ml/min. The four pH's were controlled in separate head tanks, each equipped with a pH controller (Signet Model 9030 Intel-Pro).

The size of the fish at the beginning of the test was (average $\pm$ s.d.): length = 19.4 $\pm$ 1.22 mm, range = 17-21 mm, n = 25; weight = 0.041 $\pm$ 0.0069 g, range = 0.031-0.054 g, n = 25.

Toxicity Test #4 -- larvae exposed to elevated ammonia concentrations at pH 9.5 for 30 days

We exposed larval Lost River suckers continuously to nominal unionized ammonia concentrations of 0 (control), 0.075, 0.15, 0.3, 0.6 and 1.2 mg NH_3-N/L (nominal total ammonia concentrations of 0, 0.125, 0.25, 0.5, 1 and 2 mg N/L) at pH 9.5 for 30 days, from 15 June to 15 July 1998. This exposure duration was chosen because inspection of limnological data (written

communication, Dr. Jacob Kann, Aquatic Ecosystem Sciences, Ashland, Oregon, 20 October 1997) indicated that periods of combined elevated unionized ammonia concentrations (ranging to >1 mg NH_3-N/L) and elevated pH (ranging from 9 to 10) in surface waters of Upper Klamath Lake can last for several weeks to a month. We chose pH 9.5 for these ammonia exposures because it is a commonly encountered pH in Upper Klamath Lake during summer but did not cause mortalities or sublethal effects during Toxicity Test #3. Thus, we could test the toxic effects of unionized ammonia independent of pH effects while still maintaining a realistic pH in the exposure waters. At pH 9.5 and 22 C, 59% of the total ammonia is present as unionized ammonia (NH_3, the more toxic form of ammonia to aquatic biota (Russo 1985)) and 41% is present as NH_4^+ (calculations based on equations in Emerson et al. 1975).

At the beginning of the test, we placed 18 larvae in each of 24 glass aquaria (4 replicates per each of the six ammonia concentrations). The aquaria were the same small aquaria used in Toxicity Test #3 and received water at the same flow rate (see above). However, in Toxicity Test #4, the pH was controlled at 9.5 in a single head tank. Water flowed from the head tank to a proportional diluter where a stock solution of NH_4Cl was diluted with control water to produce the five non-zero ammonia concentrations.

The size of the fish at the beginning of the test was (average $\pm$ s.d.): length = 20.6 $\pm$ 2.12 mm, range = 17-25 mm, n = 25; weight = 0.059 $\pm$ 0.0175 g, range = 0.033-0.109 g, n = 25.

Toxicity Test #5 -- early juveniles exposed to sublethal ammonia concentrations at pH 9.5 for 14 days, followed by exposure to sublethal DO concentrations for 14 days

We exposed early-juvenile Lost River suckers continuously to nominal unionized ammonia concentrations of 0 (control), 0.15 and 0.3 mg NH_3-N/L (nominal total ammonia concentrations of 0, 0.25 and 0.5 mg N/L) at pH 9.5 for 14 days, followed by one day of no ammonia at pH 8 (in transition between exposures), and then followed by continuous exposure to nominal DO concentrations of 2, 3 and 7 mg/L at pH 8 for each of the previous three ammonia exposures for 14 days, for a total 29-d exposure period from 5 August to 3 September 1998. This exposure regimen was chosen because inspection of limnological data (written communication, Dr. Jacob Kann, Aquatic Ecosystem Sciences, Ashland, Oregon, 20 October 1997) indicated that periods of combined elevated unionized ammonia concentrations (ranging to >1 mg NH_3-N/L) and elevated pH (ranging from 9 to 10) occur during cyanobacterial blooms and can last for several weeks in surface waters of Upper Klamath Lake. These cyanobacterial blooms are often followed by a "crash" of the cyanobacterial population and a concomitant combination of low DO concentrations and lower pH (both conditions associated with decomposition of the cyanobacteria) that can last for several weeks. However, to avoid trivial results, we did not test lethal combinations of pH and ammonia or lethal concentrations of DO. Instead, we tested if a dramatic shift from one almost lethal water-quality condition to a different, almost lethal water-quality condition would stress the fish enough to cause lethal or sublethal effects that weren't observed when each parameter was tested alone (i.e., in Toxicity Tests #1, #2 and #4).

At the beginning of the test, we placed 300 early juveniles in each of three large holding tanks that contained nominal unionized ammonia concentrations of 0 (control), 0.15 and 0.3 mg NH_3-N/L at pH 9.5 (i.e., one tank per each of the three sublethal ammonia concentrations). We monitored survival in those tanks for the first 14-day period (days 1-14). On day 15, we transferred 20 survivors from each sublethal ammonia concentration to each of three small aquaria (the same aquaria used in Toxicity Tests #3 and #4, see above) and allowed the fish to acclimate to the new chambers for one day, in control water containing a nominal DO

concentration of 7 mg/L and no ammonia at pH 8. Thus, a total of 36 small aquaria (4 replicates per each of the 3 DO concentrations tested for each of the 3 prior sublethal ammonia concentrations) were used during the second phase of the test. On day 16, we decreased the DO and maintained nominal concentrations of 2, 3 and 7 (control) mg/L at pH 8 for the remaining 14-day period (days 16-29).

The size of the fish at the beginning of the test was (average $\pm$ s.d.): length = 33.5 $\pm$ 1.95 mm, range = 29-37 mm, n = 45; weight = 0.331 $\pm$ 0.0550 g, range = 0.214-0.463 g, n = 45.

Endpoints for Toxicity Tests

Survival

We monitored survival several times daily, with the monitoring frequency depending on the toxicant being tested. For toxicity tests with low dissolved oxygen concentrations, fish were monitored around the clock (because staff monitored the degassing system around the clock); whereas for toxicity tests with elevated pH and elevated ammonia concentrations, fish were monitored only during normal work hours (because staff did not monitor those exposure systems at night). Dead fish were removed from the exposure aquaria, weighed, and measured as soon as they were discovered. Death was defined as the combination of cessation of operculation and lack of response when the fish were removed from the aquarium.

Growth

At the beginning of each toxicity test, we measured 25, 45 or 50 fish (25 in Toxicity Tests #3 and #4 with larvae; 45 in the Toxicity Test #5 with early juveniles; 50 in Toxicity Tests #1 and #2 with late juveniles) from the stock population for total length (nearest mm) and weighed them in a tared plastic weighing boat (nearest 0.0001 g for larvae and early juveniles; nearest 0.01 g for late juveniles). At the end of each test, all surviving fish were measured and weighed using the same techniques. Although fish that died during the test were measured and weighed, their lengths and weights were not included in the averages used in the statistical analyses of growth. Additionally, no fish that exhibited scoliosis were included in the growth analyses.

In the temperature-dependent swimming performance test, we measured (nearest mm) and weighed (nearest 0.001 g) each early-juvenile and late-juvenile fish after its swimming bout ended.

Whole-body Ion Content

We measured whole-body ion content of 5 fish per replicate aquarium in the low-dissolved-oxygen tests and 3 fish per replicate in the other tests. After being weighed and measured, the fish randomly assigned to whole-body ion analysis were refrigerated, combined in a plastic bottle, dried to constant mass at 75 C, and digested in acid at 75 C for 24 hours. In the low-dissolved-oxygen tests and the elevated-pH test, we used concentrated trace-metal-grade HNO_3 to digest the tissue; in the other two tests, we used concentrated $HClO_4$. The tissue digest was diluted to an appropriate volume (depending on tissue mass) with hydropure water and analyzed on a flame atomic absorption spectrophotometer (AAS; Perkin-Elmer Model 372) for Ca, K and Na content and on a chloridometer (Buchler-Cotlov) for Cl content. Those ion contents were normalized to a per-gram-dry-weight basis for comparisons of the treatments to the controls.

Swimming Performance

After each toxicity test, swimming performance was measured on a random subset (5 fish) of the survivors in 3 or 4 of the exposure replicates in which sufficient fish survived. The fish were swum in the same water quality to which they were exposed during the toxicity test.

Fish were transferred from the toxicity-test chamber to the swimming-performance chamber and allowed to acclimate for 5 minutes in calm water before the water velocity was incremented to the appropriate starting velocity. For Toxicity Tests #1, #3, #4 and #5, we tested swimming performance using a fixed-velocity method (McDonald et al. 1998) in which we measured endurance at the critical swimming speed (CSS, as determined in preliminary measurements using an incremental-velocity method (McDonald et al. 1998) with fish of the size being tested that were taken from our stock populations). The time to exhaustion (determined as the inability to swim away from the downstream screen in the chamber) at the CSS was recorded for each fish. For Toxicity Test #2, we used a modified version of the incremental-velocity methods of Delonay and Little (1997) and McDonald et al. (1998). For this method, we started swimming the fish at a relatively low velocity (23 cm/s = ~3 body lengths/s) and then incremented the water velocity by 2 cm/s at 1-min intervals until the fish were exhausted. The swimming speed at exhaustion was recorded for each fish.

In Toxicity Tests #1 and #2 (late juveniles), the swimming-performance apparatus was a 15-cm wide x 22.5-cm deep x 150-cm long, U-shaped plexiglass flume through which water was recirculated. A 0.5-hp motor rotated a 12.5-cm propeller that pumped the water through a 15-cm-diameter recirculation tube, and a Dart Controls Microdrive microprocessor interfaced with a Dart Controls PU-2E Hall-effect Pick-up that monitored and controlled the pump speed at the rate manually input to the microprocessor. Microprocessor settings were calibrated to water velocity in the flume using a hand-held flow meter. Screens placed 60 cm apart in the middle of the chamber prevented fish escape upstream and downstream, and for Toxicity Test #2 a 30-cm-long sheet of black plastic taped around the middle of the flume provided a refuge for the fish. Without that refuge, the fish tended to try to hide against either the upstream or downstream screen, making it (1) difficult to keep them in the main current when they hid at the perimeter of the upstream screen and (2) difficult to determine whether they were exhausted when they hid against the downstream screen.

In Toxicity Tests #3, #4 and #5 (larvae and early juveniles), the swimming-performance apparatus was a 37-mm-ID x 45-cm-long glass tube through which water flowed by gravity from a head tank. Rubber stoppers sealed each end of the tube, and water entered or exited a stopper through a hole in which a plastic nipple was inserted. Water velocity was controlled at ~15 cm/s (~167 ml/s) by adjusting the height of the outflow tubing, and the velocity was measured volumetrically for each batch of five fish that were swum together. Polyethylene screens placed at each end of the chamber prevented fish escape upstream and downstream, and a 15-cm-long sheet of black plastic taped around the middle of the flume provided a refuge for the fish (as was also needed in the larger swimming flume described above).

Gill Histopathology

We only analyzed gills collected from larvae in Toxicity Tests #3 (exposure to elevated pH) and #4 (exposure to elevated ammonia concentrations). Structural damage to the gills was assessed on a random subset (3 fish) of the survivors in each exposure replicate. Problems encountered while embedding gills from Toxicity Tests #1 and #2 (low DO concentrations) and Toxicity Test #5 (elevated ammonia concentrations followed by low DO concentrations)

precluded their analysis.

For each larva chosen for histopathology, we weighed and measured and then fixed the larva with formaldehyde and glutyraldehyde (a fixation solution of 95% 0.1 M phosphate-buffered saline at pH 7.4, 4% formaldehyde and 1% glutyraldehyde) before processing the next fish. Because of the relatively small size of the larval suckers used in the elevated-pH and the elevated-ammonia tests, we preserved and embedded the entire fish rather than just the gill arch.

After the fish was euthanized in the fixative for a few minutes, we removed its opercular flaps to allow better access to the gills. If larvae in these tests were large, we also cut off their nose and tail so their bodies would fit into embedment trays. Following this dissection, the fish were placed back into the fixative for another 3-12 hours. Removal of the opercular flaps, nose and tail additionally enhanced infiltration of fixative (and, later, resin) into the gills.

After the fixation was completed, we sequentially rinsed the fish in increasingly concentrated ethanol solutions (70%, 95%, and 100%) and then infiltrated the body with a plastic resin (Araldite 502) to embed the tissue (protocol of Dr. Pierre Laurent, University of Strasbourg, France, provided to us by Annie Bergman, Department of Zoology and Physiology, University of Wyoming). This type of fixation-and-embedding procedure produces minimal artifact and preserves cell structure better than other techniques, including paraffin embedment (Johnson and Bergman 1984).

Thin sections (2-3 μm) of lamellar tissue were cut through the embedded fish using an ultramicrotome (Sorvall Instruments Model MT-100), mounted on glass slides, and stained with Azure B, a methylene blue derivative. After first viewing the slides under a light microscope (Leitz Deluxe Microscope) to map the usable lamellar fields, we randomly selected five sampling sites per set of sections from each treatment (Mueller et al. 1991). Then we photographed high-magnification (1,000x) images of selected fields using a digital camera (Pixera Model PVC 100C) and computer software (Pixera Visual Communication Suite).

We used established morphometric and stereological methods (Weibel 1979) to quantify dimensional characteristics of the secondary lamellae. The digital images were analyzed on a high-resolution computer screen with Adobe Photo Shop 5.0 and Sigma Scan/Pro 4.0. To avoid observer bias, we employed a random sampling procedure. With a digital image projected on the computer screen, we superimposed an electronic grid on the image and directly measured lamellar thickness at the grid intersections (50-100 hit points per exposure tank; 200-400 measurements per exposure concentration). The camera was calibrated to convert image measurements (in pixels) to distance measurements (in μm). Using a similar grid overlay, minimum oxygen diffusion distance was measured at sites where grid lines intersected the outer epithelial layer of the lamella (similar to the approach of Tietge et al. 1988). Because earlier studies demonstrated that measurements of epithelial-layer thickness are highly variable due to high variation in capillary diffusion distance within a species (Newstead 1967) and the highly anisotropic nature of gill tissue (Johnson and Bergman 1984), we measured 500-1,000 diffusion distances per exposure concentration.

In addition to those quantitative measurements, we qualitatively assessed lamellar cell composition and general gill pathology. Cell hypertrophy (swelling), cell hyperplasia (increased number of cells), lamellar degeneration or necrosis (cell death), infiltration of white blood cells into the lymphatic space, and general size and distribution of cell types (mucous, chloride, pilaster, epithelial) were of particular interest.

Temperature-dependent Swimming Performance Tests

Temperature-dependent swimming performance was tested in November and December 1998. Juvenile suckers shipped to RBEBL in October 1997 were maintained in well water at RBEBL for one year and, thus, were ~1½ years old when tested; those fish are referred to as late juveniles in this report, and their size range (60-96 mm) was similar to the size range (~70-100 mm) of late-juvenile Lost River suckers tested by Delonay and Little (1997). Larval suckers shipped to RBEBL in Spring 1998 were ~6 months old when tested; those fish are referred to as early juveniles in this report, although their size range (39-64 mm) was slightly larger than the size range (12-46 mm) of early-juvenile shortnose suckers tested by Delonay and Little (1997).

Separate groups of 25 Lost River suckers in each of the two size classes (early and late juveniles) were acclimated to well water (pH 7.5-7.8, 176-202 mg/L alkalinity, 183-203 mg/L hardness, 420-477 µS/cm conductivity, 5.5-7.7 mg/L dissolved oxygen) at each of 5 nominal water temperatures (7, 10, 15, 20 and 25 C) for a minimum of one week prior to the swimming-performance tests (Table 10). Each group of fish was then swum at its acclimation temperature in well water. To minimize variations in exposure-water quality, each group of fish for a given temperature was swum within a 1- or 2-day period.

The swimming-performance apparatus was the same 15-cm wide x 22.5-cm deep x 150-cm long, U-shaped plexiglass flume that we used to test swimming performance after Toxicity Tests #1 and #2 (see Endpoints for Toxicity Tests, Swimming Performance section above).

We followed the swimming-performance procedure of Delonay and Little (1997) with the following exception: in each size class at each temperature, 25 fish were swum in 5 separate groups of 5 fish each (rather than 20 fish in 10 separate groups of 2 fish each). Prior to each trial, the fish were allowed to acclimate for 30 min in the swimming chamber before water flow started. Water velocity was increased in increments of 6.2 cm/s at 5-min intervals (same increment and interval as used by Delonay and Little 1997). The observer recorded the water velocity and amount of time at that velocity, at which each fish fatigued and became impinged on the downstream screen. After the last fish in a group fatigued, all 5 fish were blotted dry, measured (total length) and weighed (wet mass). Critical swimming speed (CSS, in cm/s) for each fish was calculated as follows (Brett 1964, Little et al. 1990, Delonay and Little 1997, McDonald et al. 1998):

$$CSS = u_{i-1} + u_{inc} \cdot \frac{t_i}{t_{max}} \qquad \text{(Eqn. 1)}$$

where u_{i-1} = water velocity maintained during the last velocity interval completed before fatigue (cm/s), u_{inc} = water-velocity increment (cm/s), t_i = amount of time the fish swam during the water-velocity interval that caused fatigue (s), and t_{max} = maximum swimming interval at each water velocity (s). In this study, u_{inc} was 6.2 cm/s and t_{max} was 300 s. In addition to expressing CSS in terms of water velocity, we calculated a body-length-normalized critical swimming speed (CSS_{BL}, in body lengths/s) for each fish, as follows:

$$CSS_{BL} = \frac{CSS}{L} \qquad \text{(Eqn. 2)}$$

where L = total length of fish (cm).

Chemical Analyses

We analyzed routine water-quality parameters regularly during the toxicity tests and the acclimation periods prior to the temperature-dependent swimming performance tests. When it was the parameter being varied, DO or pH was measured several times per day; otherwise, those parameters were monitored daily, as temperature, hardness, alkalinity and conductivity were in all of the toxicity tests. When ammonia was the parameter being varied, it was monitored daily; otherwise, it was monitored once per week. Standard methods (APHA et al. 1995) were used for alkalinity (titration), hardness (titration), pH (calibrated Beckman Model 12 pH Meter) and conductivity (calibrated Extech Digital Conductivity Meter). DO was measured with a calibrated meter (YSI Model 57), and ammonia was measured colorimetrically (Verdouw et al. 1978). We calculated unionized ammonia concentration from measured temperature, pH, and total ammonia concentration according to Emerson et al. (1975). We stopped measuring water chemistry in an aquarium when all of the fish in that aquarium died.

Statistical Analyses

Toxicity Tests

At the end of a toxicity test, the proportion of fish surviving in each replicate aquarium was transformed with the arcsine-square root function (Sokal and Rohlf 1981). Lengths, weights, whole-body ion contents, and maximum swimming speeds (for Toxicity Test #2) were not transformed before analysis. If the data were normally distributed and the variances were homogeneous, those endpoints were analyzed by analysis of variance (ANOVA) followed by one-tailed Dunnet's t-Tests (if the sample sizes were equal) or one-tailed Bonferroni t-Tests (if the sample sizes were not equal) for post hoc comparisons of all treatments with the control (Lewis et al. 1994). If either of those conditions was violated ($P < 0.01$), the data were analyzed by the appropriate non-parametric analog of ANOVA -- a one-tailed Steel's Many-one Rank Test if the sample sizes were equal or a one-tailed Wilcoxon's Rank Sum Test if the sample sizes were not equal (Lewis et al. 1994) -- for comparisons of all treatments with the control. To avoid potential bias, treatments in which survival decreased significantly were not included in analyses of length, weight, whole-body ion content, and swimming performance.

Because of (1) wide variation in the ranges of within-replicate times of swimming to exhaustion, (2) non-normal distributions of those times, and (3) frequently truncated data (e.g., observations were terminated if fish swam 20 min at the CSS), the swimming-performance data for each replicate aquarium in Toxicity Tests #1, #3, #4 and #5 were transformed to the median endurance time before being analyzed by non-parametric ANOVA (i.e., either a one-tailed Steel's Many-one Rank Test or a one-tailed Wilcoxon's Rank Sum Test). However, the inferences based on the analysis of median times to exhaustion were usually the same as those based on parametric analyses of mean times to exhaustion that we conducted for comparative purposes.

Median lethal concentrations (LC50s) were calculated from pooled survival data in all

replicates of each exposure concentration or pH. using the Trimmed Spearman-Karber method with smoothed proportions (Lewis et al. 1994).

All statistical tests for survival, length, weight, whole-body ion content, and swimming performance in the toxicity tests and the calculation of LC50s were conducted using TOXSTAT Version 3.4 (WEST, Inc. and Gulley 1994) and SPSS 8.0 for Windows. Our criterion for statistical significance was $\alpha = 0.05$ for individual ANOVAs and for the family of post hoc comparisons of treatments with a control.

Gill Histopathology Analyses

Average lamellar thicknesses and diffusion distances in gills were calculated for fish in each replicate aquarium. Because the data were normally distributed and had homogenous variances, no transformations were necessary (Chi-square Goodness of Fit Test for normality, Bartlett's Test for homogeneity of variances) and the data were analyzed by ANOVA. For Toxicity Test #3 (larvae exposed to elevated pH for 30 days), sample sizes were equal; thus, differences between the high-pH treatments and the control were tested using one-tailed Dunnett's post hoc comparisons (Ho: treatment $\leq$ control). For Toxicity Test #4 (larvae exposed to elevated ammonia concentrations at pH 9.5 for 30 days), sample sizes were not equal; thus, differences between the elevated-ammonia concentrations and the control were tested using one-tailed Bonferroni post hoc comparisons (Ho: treatment $\leq$ control). All statistical tests for gill histopathology data were conducted using TOXSTAT Version 3.4 (WEST, Inc. and Gulley 1994), with $\alpha = 0.01$.

Temperature-dependent Swimming Performance Tests

CSS and CSS_{BL} violated the assumption of homogeneity of variances for parametric analysis of variance ($P<0.001$, Levene's Test), and natural-logarithm and square-root transformations did not remove the heterogeneity ($P<0.001$ for both transformations). Therefore, the non-parametric Kruskal-Wallis Test was used to test for significant differences in CSS or CSS_{BL} among temperatures within each size class of fish at the $\alpha = 0.05$ level. If significant differences were indicated, the non-parametric Mann-Whitney Test was used for post-hoc pairwise comparisons among temperatures. Because pairwise comparisons were being made among 5 temperatures within each size class of fish, we used $\alpha = 0.01$ (i.e., 0.05/5) for the individual pairwise comparisons to ensure an overall family confidence level of 95% (Bonferroni method; Neter et al. 1990). The Mann-Whitney Test was also used to conduct pairwise comparisons between the two size classes of fish at each temperature, with $\alpha = 0.01$.

Because critical swimming speed appeared to be related to body size and temperature, least-squares multiple linear regression was used to develop predictor equations for CSS and CSS_{BL} with body size and temperature as the predictor variables.

All statistical tests for temperature-dependent swimming performance data were conducted using SPSS 8.0 for Windows.

RESULTS

Toxicity Test #1 -- late juveniles exposed to low DO concentrations (with access to the water surface) for 14 days

Averages of water quality parameters were: temperature - 22 C, pH - 8.1, hardness - 50 mg/L as $CaCO_3$, alkalinity - 51 mg/L as $CaCO_3$, conductivity - 133 µS/cm, and total ammonia - 0.08 mg N/L (Table 1). Mean DO concentrations were 1.5, 2.0, 3.0, 3.8, 4.7 and 6.3 (control) mg/L (Table 2). Variability of these DO concentrations is shown in Figure 2b.

Survival was significantly decreased only in the 1.5 mg/L exposures (33% survival in 1.5 mg/L vs. 100% survival in the controls; one-tailed Steel's Many-one Rank Test; Fig. 2a and Table 2). Because most of the mortalities occurred during the first day of the test, the LC50s for all observation times $\geq$1 day were approximately equal. For example, the 96-h LC50 was 1.57 mg/L (95% C.I. = 1.52-1.64), and the 30-d LC50 was 1.64 mg/L (95% C.I. = 1.59-1.69). DO concentrations were <1.5 mg/L for a few hours during the first day (Fig. 2b).

Scoliosis occurred in the 2.0, 3.0, 3.8 and 4.7 mg/L treatments and in the controls (Table 2), at average frequencies as high as 10% (in the 3.8 and 4.7 mg/L exposures) and at maximum frequencies in individual aquaria as high as 20% (in the 3.8 and 4.7 mg/L exposures, and in the controls). The frequency of scoliosis in the sublethal DO concentrations (2.0-4.7 mg/L) did not differ significantly from the controls (one-tailed Dunnett's t-Test, with the 1.5 mg/L treatment removed from the analysis because of significant mortality). Although no scoliosis was observed in any of the fish (alive or dead) in the 1.5 mg/L exposures, most of the deaths in this low DO concentration occurred during the first day (Fig. 2a) -- before scoliosis was observed in the other treatments and the controls.

After fish with scoliosis were excluded from the analysis, the lengths and weights of survivors in the sublethal DO concentrations (2.0-4.7 mg/L) did not differ significantly from the controls (one-tailed Dunnett's t-Tests, with the 1.5 mg/L treatment removed from the analysis because of significant mortality; Table 2). Similarly, whole-body contents of Ca, K, Na and Cl in the sublethal DO concentrations did not differ significantly from the controls (one-tailed Dunnett's t-Tests, with the 1.5 mg/L treatment removed from the analysis because of significant mortality; Table 2). Finally, swimming performance of fish in the sublethal DO concentrations did not differ significantly from the controls (one-tailed Wilcoxon's Rank Sum Test; Table 2).

Although we did not quantify this behavior with systematic observations, we observed >~75% of the survivors in the 1.5 mg/L exposures and ~20-25% of the survivors in the 2.0 mg/L exposures gulping at the surface of the water during the first few days of the test. Generally, the frequency of gulping decreased later in the test but was still relatively high in the 1.5 mg/L exposures (average of ~50% of the survivors). Because this ability to pump water that potentially contained high DO concentrations past their gills might have biased the survival of the fish in the two lowest DO concentrations, in Toxicity Test #2 we prevented the fish from having access to the water surface.

Toxicity Test #2 -- late juveniles exposed to low DO concentrations (without access to the water surface) for 14 days

Averages of water quality parameters were: temperature - 22 C, pH - 8.0, hardness - 51 mg/L

as $CaCO_3$, alkalinity - 53 mg/L as $CaCO_3$, conductivity - 121 µS/cm, and total ammonia - 0.07 mg N/L (Table 1). Mean DO concentrations were 1.4, 2.1, 3.0, 4.0, 4.9 and 6.0 (control) mg/L (Table 3). Variability of these DO concentrations is shown in Figure 3b.

Survival was significantly decreased only in the 1.4 mg/L exposures (42% survival in 1.4 mg/L vs. 100% survival in the controls; one-tailed Steel's Many-one Rank Test; Fig. 3a and Table 3). Because almost all of the mortalities occurred during the first three days of the test, the LC50s for all observation times $\geq$4 days were approximately constant. For example, the 96-h LC50 was 1.27 mg/L (95% C.I. = 1.18-1.39), and the 30-d LC50 was 1.51 mg/L (95% C.I. = 1.43-1.61). DO concentrations were always <1.5 mg/L during the first three days, before increasing to straddle 1.5 mg/L during the remainder of the test (Fig. 3b).

Scoliosis occurred less frequently than it did in Toxicity Test #1. The highest average frequency was 7% (in the 3.0 mg/L exposures; Table 3), and the maximum frequency in individual aquaria was 13% (in the 3.0 and 4.9 mg/L exposures). The frequency of scoliosis in the sublethal DO concentrations (2.1-4.9 mg/L) did not differ significantly from the controls (one-tailed Dunnett's t-Test, with the 1.4 mg/L treatment removed from the analysis because of significant mortality). Although no scoliosis was observed in any of the fish (alive or dead) in the 1.4 mg/L exposures, all of the deaths in this low DO concentration occurred during the first three days (Fig. 3a) -- before scoliosis was observed in the other treatments and the controls.

After fish with scoliosis were excluded from the analysis, the lengths and weights of survivors in the sublethal DO concentrations (2.1-4.9 mg/L) did not differ significantly from the controls (one-tailed Dunnett's t-Tests, with the 1.5 mg/L treatment removed from the analysis because of significant mortality; Table 3). Similarly, whole-body contents of K, Na and Cl in the sublethal DO concentrations did not differ significantly from the controls (one-tailed Dunnett's t-Tests, with the 1.5 mg/L treatment removed from the analysis because of significant mortality; Ca was not analyzed; Table 3). Finally, swimming performance of fish in the sublethal DO concentrations did not differ significantly from the controls (one-tailed Dunnett's t-Test; Table 3).

Toxicity Test #3 -- larvae exposed to elevated pH for 30 days

Averages of the non-varied water quality parameters were: temperature - 22 C, hardness - 54 mg/L as $CaCO_3$, DO - 5.9 mg/L, and total ammonia - 0.18 mg N/L (Table 1). Mean pH values were 8.0 (control), 9.0, 9.5 and 10.0 (Tables 4 and 5). Variability of these pH values is shown in Figure 4b. Because KOH was added to the exposure waters to increase the pH, the alkalinity and conductivity (but not the hardness) of the exposure waters increased as pH increased (average alkalinities were 53, 60, 72 and 107 mg/L as $CaCO_3$, and average conductivities were 135, 171, 166, and 261 µS/cm in the pH 8.0, 9.0, 9.5 and 10.0 treatments, respectively; Table 4). Total ammonia concentrations varied considerably and sometimes were high (up to 0.4 mg N/L) because, especially during the first week of the test before the problem was discovered, water samples for ammonia analyses were collected immediately after the fish were fed. After we changed the timing of the water sampling, the ammonia concentrations decreased but remained highly variable.

Survival did not differ significantly between any of the high-pH treatments and the controls (one-tailed Dunnet's t-Test; Fig. 4a and Table 5). Because mortality was <50% in all of the pH's, the LC50s for all observation times were greater than pH 10.0.

Scoliosis occurred less frequently than it did in Toxicity Test #1, but at approximately the same frequency as in Toxicity Test #2. The highest average frequency was 6% (in the pH 9.0 exposures; Table 5), and the maximum frequency in individual aquaria was 17% (in the pH 9.0 exposures). The frequency of scoliosis in the high-pH treatments (pH 9.0-10.0) did not differ significantly from the controls (one-tailed Dunnett's t-Test).

After fish with scoliosis were excluded from the analysis, the lengths and weights of survivors in the high-pH treatments (pH 9.0-10.0) did not differ significantly from the controls (one-tailed Dunnett's t-Tests; Table 5). Similarly, whole-body contents of Ca, K, and Cl in the high-pH treatments did not differ significantly from the controls (one-tailed Dunnett's t-Tests; Table 6); but whole-body content of Na in pH 10.0 was significantly lower than in the controls (a 14% decrease; Table 6). Swimming performance of fish in the high-pH treatments did not differ significantly from the controls (one-tailed Wilcoxon's Rank Sum Test; Table 6), probably because of a small sample size (n = 3 or 4) and high within-treatment variability. However, no fish from the pH 10.0 treatment swam long at the critical swimming speed of 4.8 body lengths/sec, whereas many fish from the controls and pH 9.0 aquaria swam at that speed for 20 min (the maximum time the fish were swum). Thus, with larger sample sizes, swimming performance of the pH 10.0 fish might have been significantly decreased relative to the controls.

Neither of the quantified gill-structure parameters (mean lamellar thickness and mean lamellar diffusion distance) in the high-pH treatments differed significantly from the controls (one-tailed Dunnett's t-Tests; Table 5). Additionally, our qualitative observations indicated no differences in lamellar cell composition (e.g., numbers of mucous, chloride, pilaster, and epithelial cells) or gill pathology (e.g., cell hyperplasia and hypertrophy; tissue degeneration and necrosis; and infiltration of white blood cells into the lymphatic space).

Toxicity Test #4 -- larvae exposed to elevated ammonia concentrations at pH 9.5 for 30 days

Averages of water quality parameters were: temperature - 22 C, pH - 9.5, hardness - 51 mg/L as $CaCO_3$, alkalinity - 71 mg/L as $CaCO_3$, conductivity - 162 µS/cm, and DO - 6.2 mg/L (Table 1). Mean unionized ammonia concentrations were 0.01 (control), 0.11, 0.20, 0.37, 0.69 and 1.16 mg NH_3-N/L (Table 6); the corresponding mean total ammonia concentrations were 0.01 (control), 0.18, 0.33, 0.63, 1.23 and 2.30 mg N/L, respectively. Variability of these unionized ammonia concentrations is shown in Figure 5b.

Survival was significantly decreased in the 0.69 and 1.16 mg NH_3-N/L exposures (1% survival in 0.69 mg/L and 0% survival in 1.16 mg/L vs. 92% survival in the controls; Steel's Many-one Rank Test; Fig. 5a and Table 6). Most of the mortalities in the 1.16 mg NH_3-N/L exposures occurred during the first three days of the test, whereas the mortalities in the 0.69 mg NH_3-N/L exposures occurred gradually from days 3 to 24. Mortality averaged 29% in the 0.37 mg NH_3-N/L exposures; however, almost all of this was due to 100% mortality that occurred in one of the four replicate aquaria during a 3-day period (days 5 to 7; Fig. 5a). Although this might indicate an exogenous perturbation (e.g., accidental introduction of a toxicant to or a disease outbreak in only that aquarium), we recorded no abnormal water chemistry and observed no external signs of disease in those fish. Thus, we have included that replicate in the data analyses, despite its suspicious appearance.

With all replicates included, the 96-h LC50 was 0.91 mg NH_3-N/L (95% C.I. = 0.88-0.93);

and the 30-d LC50 was 0.45 mg NH_3-N/L (95% C.I. = 0.42-0.48). With the 100%-mortality replicate at 0.37 mg/L excluded, the 96-h LC50 was 0.91 mg NH_3-N/L (95% C.I. = 0.88-0.93); and the 30-d LC50 was 0.52 mg NH_3-N/L (95% C.I. = 0.51-0.54).

Although scoliosis generally occurred less frequently than it did in Toxicity Test #1, average frequencies were as high as 10% (in the controls; Table 6) and maximum frequencies in individual aquaria were as high as 21% (in a 0.11 mg NH_3-N/L exposure). The frequency of scoliosis in the sublethal ammonia concentrations (0.11-0.37 mg NH_3-N/L) did not differ significantly from the controls (Steel's Many-one Rank Test, with the 0.69 and 1.16 mg NH_3-N/L treatments removed from the analysis because of significant mortality). No scoliosis was observed in any of the fish (alive or dead) in the 0.69 and 1.16 mg NH_3-N/L exposures, in part because most of the deaths in these high ammonia concentrations occurred before scoliosis was observed in the other treatments and the controls.

After fish with scoliosis were excluded from the analysis, the lengths and weights of survivors in the sublethal ammonia concentrations (2.0-4.7 mg/L) did not differ significantly from the controls (one-tailed Bonferroni t-Tests, with the 0.69 and 1.16 mg NH_3-N/L treatments removed from the analysis because of significant mortality; Table 6). Similarly, whole-body contents of Ca, K, Na and Cl in the sublethal ammonia concentrations did not differ significantly from the controls (one-tailed Bonferroni t-Tests, with the 0.69 and 1.16 mg NH_3-N/L treatments removed from the analysis because of significant mortality; Table 6). Finally, swimming performance of fish in the sublethal ammonia concentrations did not differ significantly from the controls when analyzed using non-parametric statistics (one-tailed Wilcoxon's Rank Sum Test on median times to exhaustion at critical swimming speed; Table 6), but swimming performance of the fish exposed to 0.11 mg NH_3-N/L was significantly greater than the controls when analyzed using parametric statistics (two-tailed Bonferroni t-Test on average times to exhaustion at critical swimming speed; results not shown). However, because of the unequal variances among treatments and non-normal distributions of the swimming speeds within aquaria, the non-parametric analysis was more appropriate.

Both of the quantified gill-structure parameters (mean lamellar thickness and mean lamellar diffusion distance) increased in some of the sublethal ammonia concentrations (Figs. 6 and 7). Mean lamellar thickness was significantly greater in the 0.37 mg NH_3-N/L treatment than in the controls (a 32% increase), and mean lamellar diffusion distance was significantly significantly greater in the 0.20 and 0.37 mg NH_3-N/L treatments than in the controls (37% and 76% increases, respectively; one-tailed Bonferroni t-Tests; Table 6). Qualitatively, we also observed a trend of increased numbers of chloride and mucous cells, the appearance of mitotic figures, and infiltration of white blood cells into the lymphatic space, in fish exposed to increasingly higher ammonia concentrations (Figs. 7 and 8). In addition, pillar cells (a.k.a. pilaster cells) appeared to be slightly longer in gills of control fish, but might have only appeared to be longer in comparison to the shorter lamellar diffusion distance in the control fish.

Toxicity Test #5 -- early juveniles exposed to sublethal ammonia concentrations at pH 9.5 for 14 days, followed by exposure to sublethal DO concentrations for 14 days

Averages of the non-varied water quality parameters were: temperature - 22 C, and hardness - 49 mg/L as $CaCO_3$ (Table 1). Mean pH during the sublethal ammonia-exposure phase (days 1-14) was 9.5 and during the sublethal DO-exposure phase (days 16-29) was 8.0 (Tables 7 and 8).

Mean DO concentrations during the sublethal ammonia-exposure phase were ~6.0 mg/L and during the sublethal DO-exposure phase were ~2.0, ~3.0 and ~5.8 mg/L (Tables 7 and 8). Mean unionized ammonia concentrations during the sublethal ammonia-exposure phase were 0.01, 0.17 and 0.30 mg NH_3-N/L and during the sublethal DO-exposure phase were $\leq$0.001 mg/L (Tables 7 and 8). Variability of these pH's and DO and ammonia concentrations is shown in Figure 9. Because KOH was added to the exposure waters to increase the pH, the alkalinity and conductivity (but not the hardness) of the exposure waters increased as pH increased (average alkalinities ranged from 44 to 65 mg/L as $CaCO_3$, and average conductivities ranged from 111 to 171 µS/cm; Tables 7 and 8).

Survival was >99% in all three sublethal ammonia concentrations (days 1-14). During the sublethal DO-exposure phase (days 16-29), survival was $\geq$89% and did not differ significantly in any of the ammonia/DO treatments (relative to the 0 mg/L ammonia:7 mg/L DO controls; one-tailed Steel's Many-one Rank Test; Table 9). LC50s could not be calculated because mortality did not exceed 50% in any treatment. All of the deaths during the sublethal DO-exposure phase occurred in the 2 mg/L DO exposures in all three ammonia pretreatments, and almost all occurred during the first two days.

Scoliosis was not observed during this test.

None of the lengths and weights of survivors in the ammonia/DO treatments differed significantly from the 0 mg/L ammonia:7 mg/L DO controls (one-tailed Dunnett's t-Tests; Table 9). Similarly, whole-body contents of Ca, K, Na and Cl in the ammonia/DO treatments did not differ significantly from the 0 mg/L ammonia:7 mg/L DO controls (one-tailed Dunnett's t-Tests for Ca and K, and one-tailed Steel's Many-one Rank Tests for Na and Cl; Table 9). Finally, swimming performance of fish in the ammonia/DO treatments did not differ significantly from the 0 mg/L ammonia:7 mg/L DO controls (one-tailed Steel's Many-one Rank Test on median times to exhaustion at critical swimming speed; Table 9).

Temperature-dependent Swimming Performance Tests

Average water-quality conditions (ranges of values in parentheses) in the well water over the two-month testing period were: pH - 7.7 (7.6-7.9); alkalinity - 187 (168-200) mg/L as $CaCO_3$; hardness - 189 (164-203) mg/L as $CaCO_3$; conductivity - 439 (389-462) µS/cm; and DO - 6.6 (5.9-7.4) mg/L (Table 10). Average sizes of the early juveniles tested at the five temperatures ranged from 47.0-49.6 mm and 0.77-0.88 g, and the overall ranges of sizes were 39-64 mm and 0.40-1.97 g; average sizes of the late juveniles at the five temperatures ranged from 73.8-77.9 mm and 3.58-4.03 g, and the overall ranges of sizes were 60-96 mm and 2.02-7.20 g (Table 11).

For the late juveniles, CSS increased significantly as temperature increased but appeared to be approaching an asymptote at the higher temperatures (Fig. 10 and Table 11). Although the same trend was apparent for early juveniles, CSS at 7 C was significantly higher than at 10 C for unknown reasons.

For early juveniles, the lowest 10%-ile CSS (i.e., the water velocity that was exceeded by the CSS of 90% of the fish at a specified temperature) was 14.9 cm/s (at 10 C), and the highest 10%-ile CSS was 22.7 cm/s (at 20 C); whereas for late juveniles, the lowest 10%-ile CSS was 14.2 cm/s (at 7 C), and the highest 10%-ile CSS was 26.9 cm/s (at 20 C) (Table 11).

At 10, 15 and 25 C, late juveniles swam significantly faster than early juveniles ($P\leq0.005$); but at 7 C the late juveniles swam significantly slower than the early juveniles ($P<0.001$), and at

10 C the difference in CSS was not significant (P=0.308). However, the early juveniles swam significantly more body lengths per unit time than did the late juveniles at all five temperatures (Fig. 11 and Table 11; $P \leq 0.006$).

DISCUSSION

Toxicity Tests

DO Tests

Results of our two 14-d toxicity tests with late-juvenile Lost River suckers exposed to low DO concentrations are consistent with the 96-h tests conducted by Saiki et al. (1999) with larval and younger juvenile Lost River suckers (Table 12). Our 24-, 48- and 96-h LC50s were slightly lower than those for the smaller juveniles tested by Saiki et al. (1999), consistent with the pattern of decreasing sensitivity to low DO (i.e., decreasing DO LC50s) as body size increased from larvae to juveniles in their tests (Table 12). Similar to Saiki et al.'s (1999) results, most of the mortalities in our tests occurred within the first 24 hours. Thus, our 30-d LC50s were only slightly higher than our 96-h LC50s.

Our results are also consistent with those of Martin and Saiki (1999), who reported high mortality of caged juvenile Lost River suckers when DO concentrations in Upper Klamath lake temporarily decreased below 1.4 mg/L; whereas few mortalities occurred in their study when DO concentrations were $\geq$1.58 mg/L. Most of the mortalities in our first DO test occurred during the first day, when DO concentrations were <1.5 mg/L (Fig. 2); and all of the mortalities in our second DO test occurred during the first four days, when DO concentrations were $\leq$1.5 mg/L (Fig. 3).

Surprisingly, the three sublethal toxicity endpoints we tested (growth, whole-body ion content, and swimming performance) were not significantly affected at the lowest DO concentration in which survival was not significantly affected (~2 mg/L; Table 12). In fact, there was not even a trend toward decreased growth -- a generally expected response to low-DO stress -- until the lethal DO concentrations (1.44 and 1.54 mg/L) were encountered (Tables 2 and 3).

Fish in the two lowest DO concentrations in the first test spent considerable time gulping at the water surface. Thus, the results of that test might have been biased because the fish that gulped surface water might not have experienced the low DO concentrations we measured deeper in the water column. Although we expected decreased tolerance of low DO concentrations in the second test (because we prevented the fish from having access to the water surface), the results were almost identical to the first test. Therefore, either (1) the surface water the fish gulped during the first test did not contain appreciably higher DO concentrations than the deeper water, or (2) the surface water the fish gulped during the first test contained higher DO concentrations, but the benefits of inspiring it were offset by the energetic cost of the gulping activity. Most importantly, the results of the first test should be interpreted cautiously, because of the relatively high frequency of scoliosis (Table 2).

pH Test

Although the highest pH we tested (pH 10.0) did not cause significant mortalities, the results of our 30-d toxicity test with larval Lost River suckers exposed to elevated pH are consistent with the 96-h tests conducted by Saiki et al. (1999) with larval and juvenile Lost River suckers and shortnose suckers (Table 13). In all of their acute-toxicity tests, the LC50s were $\geq$10.3.

The only sublethal effect we observed was a 14% decrease in whole-body Na content at pH 10.0. To place this Na loss in perspective, Freda and McDonald (1988) reported $\geq$~50% decreases of whole-body ion contents at death in three fish species exposed to lethal low pH for 4-25 hours (49% and 57% losses of Na and Cl, respectively, in rainbow trout (*Oncorhynchus*

mykiss); 53% and 63% losses in common shiners (*Notropis cornutus*); and 58% and 71% losses in yellow perch (*Perca flavescens*)). However, because we did not measure whole-body ion content of our Lost River suckers during the first few days of exposure to the elevated pH's, we do not know if (1) the Na losses never exceeded 14% or (2) the Na losses were greater during the early part of the exposure but then decreased as the fish acclimated to the high pH.

Surprisingly, growth, swimming performance and gill structure (tested at the pH maintained during the 30-d exposure) were not significantly impaired at pH 10.0. However, there was a trend toward shorter times to exhaustion at the critical swimming speed in the fish exposed to pH 10.0; thus, with a larger sample size (i.e., more than 4 replicate aquaria per pH), the swimming performance at pH 10.0 might have been significantly lower than in the pH 8.0 controls (Table 5).

Although we also attempted to test the effects of pH 10.5 on Lost River suckers, rapid and massive precipitation of $CaCO_3$ in our diluter system precluded testing that pH. To circumvent that problem in a test lasting longer than a few days, we would have had to conduct the test in water with much lower hardness (i.e., much lower than the 50 mg/L as $CaCO_3$ hardness used in all of our tests).

Ammonia Test

During the first four days of exposure, the Lost River sucker larvae exposed to ammonia in our 30-d test were less sensitive (i.e., they had higher LC50s) than the Lost River sucker larvae tested by Saiki et al. (1999) (Table 14). However, by the end of the 30-d test, the unionized ammonia LC50 for our larvae (0.45 mg NH_3-N/L) was approximately the same as the 96-h LC50 for Saiki et al.'s (1999) larvae (0.48 mg NH_3-N/L). Because the LC50s reported by Saiki et al. (1999) appeared to have been approaching an asymptote by 96 hours, the results of both studies are consistent with an incipient lethal level (i.e., the LC50 for infinite exposure time) of ~0.4-0.5 mg NH_3-N/L.

Surprisingly, the three traditional sublethal toxicity endpoints we tested (growth, whole-body ion content, and swimming performance) were not significantly affected at the highest unionized ammonia concentration in which survival was not significantly affected (0.37 mg NH_3-N /L; Tables 6 and 14). In fact, there was not even a trend toward decreased growth -- a generally expected response to ammonia stress -- in the sublethal concentrations (Table 6). However, gill structure -- a less traditional sublethal endpoint -- changed significantly in fish exposed to the two highest sublethal ammonia concentrations (see Gill Histopathology section below). The large decreases in length and weight in the next-higher concentration (0.69 mg NH_3-N /L) should be interpreted cautiously, because they are based on the only fish that survived at that concentration.

Ammonia probably has different modes of action at different concentrations (Russo 1985). At high concentrations, a direct effect on the central nervous system usually results in an increase in gill ventilation, hyper-excitability, and, ultimately, death. At lower concentrations, there may not be a drastic and immediate survival effect; however, decreased reproductive capacity, deleterious histological effects, decreased growth or morphological development, and an increased susceptibility to disease may result from chronic, sublethal exposure (Smart 1976, Russo 1985, Lang et al. 1987). Thus, the absence of a growth effect in the highest sublethal ammonia concentration is difficult to explain unless Lost River suckers have evolved physiological mechanisms to tolerate high sublethal ammonia concentrations that are common in Upper Klamath Lake.

Ammonia/DO Test

Periods of high pH, high ammonia concentrations, and saturated or supersaturated DO concentrations during cyanobacterial blooms can be followed rapidly by periods of circumneutral pH and low DO concentrations during cyanobacterial "crashes" in Upper Klamath Lake. Although we speculated that such rapid water-quality changes might be detrimental, combinations of a 14-d exposure to sublethal ammonia concentrations (0.15 or 0.3 mg NH_3-N/L) at 6 mg DO/L and pH 9.5 followed by a 14-d exposure to sublethal DO concentrations (2 or 3 mg/L) at pH 8 with no ammonia caused no lethal or sublethal effects on early-juvenile Lost River suckers. Higher ammonia or DO concentrations probably would have caused deaths within either 14-d period, because significant mortality occurred within that time period at 1.5 mg/L in the low-DO tests and at 0.69 mg NH_3-N/L in the elevated-ammonia test we conducted earlier in the study. Thus, testing combinations that included these more extreme water-quality conditions would not have provided new information.

Traditional Chronic Toxicity Endpoints

Of the three traditional chronic-toxicity endpoints we used (decreased growth, loss of body ions, and decreased swimming performance), decreased growth and loss of body ions were stable indices with relatively low variability (i.e., coefficients of variation (standard deviation ÷mean) among replicate aquaria usually were <20%). However, swimming performance as measured by the fixed-velocity method (used in Toxicity Tests #1, #3, #4 and #5) did not produce satisfactory results. Endurance time at the critical swimming speed usually varied widely among the fish in a given aquarium, sometimes with some fish not being able to swim at that speed for more than a few seconds whereas other fish from the same aquarium might sustain that speed for >20 min (our maximum observation time). This forced us to analyze the results of the fixed-velocity tests using non-parametric statistics, resulting in a potential loss of statistical power relative to analogous parametric methods. On the other hand, results of the one test (#2) in which we used an incremental-velocity method were much better behaved and could be analyzed using parametric statistics. Moreover, coefficients of variation for maximum swimming speed among replicate aquaria were <15%. Thus, an incremental-velocity test appears to be superior to a fixed-velocity test for testing differences in swimming performance among treatments in a toxicity test.

Contrary to the common expectation for fish chronically exposed to toxicants, Lost River suckers generally did not display sublethal responses to low DO concentrations, elevated pH, or elevated ammonia concentrations, based on the three traditional chronic-toxicity endpoints we used. In the two 14-day exposures to low DO concentrations and in the 30-day exposure to elevated ammonia concentrations, the traditional sublethal endpoints were no more sensitive than survival was; only in the 30-day exposure to elevated pH was a sublethal endpoint (whole-body ion content) more sensitive than survival was. Although large increments between exposures can cause an apparent absence of sublethal responses in a chronic toxicity test, we had relatively narrow increments between the effects and no-effects exposures. In the DO tests, the gap between the highest effects and lowest no-effects concentration was ~0.5 mg/L (Tables 2, 3 and 12); in the pH test, the gap between the highest no-effects and lowest effects pH was 0.5 pH units (Tables 5 and 13); and in the ammonia test, the gap between the highest no-effects and lowest effects concentration was 0.32 mg NH_3-N/L (i.e., a factor of 1.9 between 0.37 and 0.69 NH_3-N/L; Tables 6 and 14).

Most likely, we would have observed sublethal effects if the increments between exposure concentrations or pH's in our tests had been even smaller. Additionally, some of the non-significant sublethal responses might have been statistically significant if we had used larger sample sizes (e.g., swimming performance at pH 10; Table 5). However, designing the tests with narrower exposure increments would have greatly increased the probability that we would not have captured effects and no-effects exposures in the same test (given the usual study design of 5 treatments and a control); and increasing the number of replicate chambers would have increased the costs of the tests or decreased the number of treatments per test (thus decreasing the probability of capturing effects and no-effects concentrations in the same test), or would have exceeded physical constraints of our exposure systems (e.g., the degassing capacity of our DO-removal system was already maximized in the two low-DO tests).

But from a management perspective, the exposure increments in our toxicity tests probably were narrow enough to help establish targets for remediation of water quality in Upper Klamath Lake. In general, Lost River suckers did not display major *functional* physiological impairment at concentrations in which no significant mortalities occurred (i.e., $\geq$2.0 mg DO/L, $\leq$ pH 10.0, and $\leq$ 0.37 mg NH_3-N/L). However, structural changes occurred in the gills of Lost River suckers at lower ammonia concentrations (see Gill Histopathology section below), suggesting that tissue damage can occur at sublethal exposure conditions and might be a useful index of acceptable water quality -- whether or not the tissue damage is severe enough to functionally impair the functioning of the fish.

From a management perspective, the exposure increments in our toxicity tests probably were narrow enough to help establish targets for remediation of water quality in Upper Klamath Lake. In general, Lost River suckers did not display major *functional* physiological impairment at concentrations in which no significant mortalities occurred (i.e., $\geq$2.0 mg DO/L, $\leq$ pH 10.0, and $\leq$ 0.37 mg NH_3-N/L). However, structural damage to gill tissue can occur at sublethal exposure conditions and might be a useful sublethal index of acceptable water quality for in-lake monitoring -- whether or not the tissue damage is severe enough to impair the functioning of the fish.

Gill Histopathology

Gills are one of the most sensitive of the exposed surfaces in fish (Daye and Garside 1976). Many studies have shown gill damage as a result of exposure to toxicants (e.g., Johnson 1983, Post 1987), including decreased pH (Daye and Garside 1976, Chevalier et al. 1985, Tietge et al. 1988, Mueller et al. 1991) and elevated ammonia concentrations (Smart 1976, Lang et al. 1987). Although we expected that the toxicants tested in our study would cause similar histopathological changes, we did not observe structural changes in gills of larval Lost River suckers that were exposed for 30 days to sublethal pH's as high as 10 (Table 5). Instead, we only detected statistically significant structural changes in gills of larval Lost River suckers exposed to elevated ammonia concentrations for 30 days (Table 6). Because of technical difficulties, we were not able to analyze the gills of juvenile Lost River suckers exposed to low DO concentrations (Toxicity Tests #1 and #2) and to sublethal ammonia concentrations followed by sublethal DO concentrations (Toxicity Test #5).

In the ammonia test, we quantified statistically significant structural gill changes at the two highest sublethal concentrations of unionized ammonia -- concentrations in which no other sublethal responses were detectable at the resolution of the traditional chronic-toxicity endpoints

we used (i.e., growth, whole-body ion content, and swimming performance; Table 6). Most notably, mean diffusion distance across the secondary lamellae was almost doubled in tissue exposed to the highest sublethal ammonia concentration (0.37 mg NH_3-N/L), relative to control tissue. Qualitatively, we frequently observed several factors that contributed to the significantly increased diffusion distances in gills exposed to 0.20 and 0.37 mg NH_3-N/L. These factors included (1) epithelial cell hypertrophy (swelling) and hyperplasia (increased number of cells), (2) mucous cell hypertrophy, and (3) swelling of the lymphatic space between the double epithelial cell layers lining the vascular spaces in the secondary lamellae. Lymphatic swelling was often accompanied by increased numbers of white blood cells, possibly indicating an immune response. Hyperemia (swelling of tissues through pressure of excess blood) might also have contributed to the increased lamellar thickness in stressed tissue. The presence of mitotic figures, most often seen in inter-lamellar regions on the primary filaments and towards the tips of the filaments, might have been caused by an increased rate of cell differentiation needed to repair damaged tissue.

The secondary lamellae of gill filaments are the primary site of oxygen uptake, ammonia excretion and ion regulation in fish (Maetz 1973). Because the epithelium of the gill is the first site in the respiratory chain that limits a fish's capacity for oxygen exchange (Laurent 1989, Morgan and Tovell 1973), damage to the gills might adversely affect the overall fitness of the fish. The relatively thin lamellar epithelium is structurally adapted for respiratory gas transfer (Laurent 1989). Increasing lamellar thickness is thus an index of decreased respiratory sufficiency (Tietge et al. 1988). Moreover, the thickness of the blood-to-water pathway provides diffusional resistance to gas exchange and affects the conditions of perfusion and ventilation on either side of the exchange surface (Hughes and Morgan 1973). Minimum oxygen diffusion distance is an index of the thickness of the blood-to-water pathway. Therefore, the increased diffusion distances in gills of Lost River suckers exposed to the two highest sublethal ammonia concentrations indicate that those fish might have been physiologically stressed -- or on the verge of being stressed -- even though traditional indicators of stress (i.e., growth, whole-body ion content, and swimming performance) at the same exposure concentrations did not differ significantly from the controls.

Mucus on gill surfaces is also thought to impede oxygen and ion diffusion in fish (Smart 1976, Ultsch and Gros 1979, Powell et al. 1994). Although we did not quantify mucus production in our toxicity tests, the hypertrophy of lamellar mucous cells in the highest sublethal ammonia concentrations might have been associated with increased mucus secretion -- potentially further impairing the respiratory and ionoregulatory capacity of the larval suckers. Supporting this, Smart (1976) reported increased mucus production on gills of rainbow trout exposed to elevated ammonia concentrations.

Surprisingly, chronic exposure to pH's as high as 10 did not appear to alter gill morphology of larval Lost River suckers. Although the structural changes observed in chronic exposure to elevated ammonia concentrations might have been synergistically affected by the high pH of 9.5 that we maintained during the ammonia exposures, the primary effect of that high pH probably was to shift the ammonia equilibrium to the more toxic, unionized (NH_4^+) form (Russo 1985). We are not aware of any published studies of chronic exposures of fish to elevated pH alone, to which our results can be compared.

Although we have discussed the potential for structural changes in the gills of Lost River suckers to physiologically impair the fish, structural changes can be compensated for and lead to no adverse effects. Because it is important to correlate structural responses to functional

responses in toxicity tests (Johnson and Bergman 1984), the absence of *functional* physiological consequences in Lost River suckers exposed to ammonia concentrations in which we observed gill damage might be interpreted to indicate that the structural changes were of no consequence to the fish. On the other hand, the traditional sublethal toxicity endpoints could have lacked adequate resolution compared to the structural indices we used in this study.

Either way, our results suggest that gill histopathology might be a useful tool to monitor fish health in Upper Klamath Lake, where elevated ammonia concentrations are linked to the eutrophic conditions that also produce high pH's and low DO concentrations at various times in summer. For example, structural changes in gills could be used as an index of fish response to lake remediation. Absence of gill structural changes in fish collected from the lake (compared, for example, to gills of hatchery-reared fish) might be used as a criterion for acceptable water quality. This could be an especially useful management tool because water quality in Upper Klamath Lake varies considerably on a daily, weekly and monthly basis, thus making results of toxicity tests conducted under constant-exposure conditions (and even most time-variable exposure regimens) difficult to interpret in light of the time-varying water quality -- even if extensive water-quality data sets have been collected. In essence, the gill could be used to temporally integrate the effects of non-constant water quality. Although the presence of gill structural changes might not necessarily mean fish are being adversely affected by the water quality in the lake, it would at least indicate the presence of ammonia concentrations that are on the verge of causing sublethal physiological impairment or death.

Temperature-dependent Swimming Performance Tests

The range of CSSs for late juveniles in our study was similar to the range of CSSs for late juvenile Lost River suckers at 17 C reported by Delonay and Little (1997; compare CSSs in Fig. 5 in their report to CSSs for late juveniles at 15 and 20 C in Fig. 10 in this report); our CSSs for early juveniles cannot be compared to Delonay and Little (1997) because they did not test early-juvenile Lost River suckers.

Because larger suckers tended to swim faster than smaller suckers when CSS was expressed as cm/s (but they tended to swim slower when CSS was expressed as body lengths/s), some of the high variability in CSS and CSS_{BL} within a group of fish at a given temperature was due to the range of body sizes tested. When results for all fish (early and late juveniles) tested at a given temperature were combined, some of the variability in critical swimming speed could be accounted for by body size; i.e., CSS tended to increase linearly as body length increased, and CSS_{BL} decreased linearly as body length increased (Fig. 12 and Table 15).

Thus, the following multiple-regression equations can be used to predict critical swimming speeds:

$$CSS = 0.131 \cdot L + 0.574 \cdot T + 7.732 \quad (R^2 = 0.464,\ P < 0.001) \qquad \text{(Eqn. 3)}$$

$$CSS_{BL} = -0.0441 \cdot L + 0.0916 \cdot T + 5.448 \quad (R^2 = 0.546,\ P < 0.001) \qquad \text{(Eqn. 4)}$$

where CSS is in cm/s, CSS_{BL} is in body lengths/s, L = body length (mm), and T = water

temperature (C). Standard errors for the three constants in Equation 3 were 0.020, 0.046 and 1.420, respectively; and standard errors for the three constants in Equation 4 were 0.003, 0.008 and 0.239, respectively. The relatively high uncertainty in those constants will produce relatively high uncertainty in estimated critical swimming speeds.

Although nonlinear predictor equations might describe trends at small and large body sizes and at low and high temperatures better than do these linear equations, the high variability within and among temperatures in our results makes it difficult to justify using nonlinear models within the range of body sizes and temperatures tested. However, because (1) critical swimming speed probably will approach a plateau as body size increases and (2) critical swimming speed probably will begin decreasing as lethal high temperatures are approached, the linear Equations 3 and 4 should only be cautiously extrapolated beyond the range of body sizes (~40-100 mm) and temperatures (7-25 C) that we tested.

CONCLUSIONS

Toxicity Tests

Lost River suckers are unusual, because they appear to have a narrow range over which sublethal effects of exposure to low DO concentrations, elevated pH, and elevated ammonia concentrations occur. Despite maintaining relatively narrow increments (0.5 mg DO/L, 0.5 pH units, and 0.32 mg NH_3-N/L) between the effects and the no-effects exposure conditions for survival in these toxicity tests, we were able to identify a significant sublethal *functional* effect of a toxiciant in only one of the three tests -- whole-body Na content decreased significantly in larvae exposed to 0.37 mg NH_3-N/L in the elevated-ammonia test. Thus, within the resolution of the traditional chronic-exposure endpoints we used in these tests, a Lost River sucker essentially had to die before an adverse *functional* effect of the toxiciant could be identified.

The relative lack of response of traditional sublethal *functional* indicators of toxicity in Lost River suckers implies that monitoring of such physiological indices would be relatively unproductive as an indicator of water quality and fish health in Upper Klamath Lake. However, quantitative gill histopathology might be a useful index (at least of exposure to elevated ammonia concentrations) because statistically significant structural changes occurred in gills of larvae exposed continuously to unionized ammonia concentrations 3.5 times lower than the lowest concentration at which significant mortality occurred. Moreover, structural damage to gills presumably would not change quickly if ammonia concentrations fluctuated frequently; instead, the structural damage to the gills would integrate the fluctuating exposures and indicate the overall responses of fish to days, weeks or months of time-varying water quality. Thus, gill histopathology could be used as a management tool to judge the success of water quality remediation in Upper Klamath Lake, assuming that general decreases in ammonia concentrations (presumably the major toxicant in the lake that causes gill structural changes) will also be accompanied by general decreases in maximum pH and increases in minimum DO concentrations.

Temperature-dependent Swimming Performance Tests

Body size and water temperature affect the critical swimming speed of juvenile Lost River suckers. Equations 3 and 4 can be used to predict differences in critical swimming speed for Lost River suckers of various body sizes and at various water temperatures within the ranges tested (~40-100 mm and 7-25 C). Such differences in swimming speed could be an important management consideration if water is pumped from Upper Klamath Lake at different times of the year when fish size and water temperature differ.

LITERATURE CITED

American Public Health Association, American Water Works Association, and Water Environment Federation (APHA et al.). 1995. Standard Methods for the Examination of Water and Wastewater. 19th Ed. APHA, Washington, DC.

Bellerud, B. and M.K. Saiki. 1995. Tolerance of Larval and Juvenile Lost River and Shortnose Suckers to High pH, Ammonia Concentration, and Temperature, and to Low Dissolved Oxygen Concentration. National Biological Service, California Pacific Science Center, Dixon Field Station, Dixon, CA.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can.* 21:1183-1226.

Camp Dresser & McKee Inc., The Cadmus Group, Habitech Inc., and Western Ecosystems Technology Inc. 1994. South Platte River Segment 15 Studies. Volume 4: Laboratory Studies to Determine the Response of Fish Species to Diel Fluctuations in Dissolved Oxygen. Report to Metro Wastewater Reclamation District, Denver, CO.

Chevalier, G., L. Gauthier and G. Moreau. 1985. Histopathological and electron microscopic studies of gills of brook trout, *Salvelinus fontinalis*, from acidified lakes. *Can. J. Zool.* 63:2062-2070.

Daye, P. G. and E.T. Garside. 1976. Histopathologic changes in surficial tissues of brook trout, *Salvelinus fontinalis* (Mitchill), exposed to acute and chronic levels of pH. *Can. J. Zool.* 54:2140-2155.

DeLonay, A.J. and E.E. Little. 1997. Swimming Performance of Juvenile Shortnose Suckers (*Chasmistes brevirostris* Cope) and Lost River Suckers (*Deltistes luxatus* Cope). Project Completion Report. Department of the Interior, U.S. Geological Survey, Biological Resources Division, Midwest Science Center, Columbia, MO.

Emerson, K., R.C. Russo, R.E. Lund and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Board Can.* 32:2379-2383.

Falter, M.A. and J.J. Cech, Jr. 1991. Maximum pH tolerance of three Klamath Basin fishes. *Copeia* 1991:1109-1111.

Foott, J. S. 1996. Results of histological examination of sucker tissues. Case 96-96. U.S. Fish and Wildlife Service. Correspondence to Mark Buettner, Bureau of Reclamation, Klamath Falls, OR.

Freda, J. and D.G. McDonald. 1988. Physiological correlates of interspecific variation in acid tolerance in fish. *J. Exp. Biol.* 136:243-258.

Hughes, G.M. and M. Morgan. 1973. The structure of fish gills in relation to their respiratory function. *Biol. Rev.* 48:419-475.

Johnson, R.D. 1983. The Use of Quantitative Histopathology in Aquatic Toxicology: Correlations between Gill Structure and Routine Toxicity Measures in Rainbow Trout. Ph.D. dissertation, University of Wyoming, Laramie, WY.

Johnson, R. D. and H.L. Bergman. 1984. Use of histopathology in aquatic toxicology: a critique. pp. 19-36 in: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects on Fisheries. John Wiley and Sons, Inc., New York, NY.

Kann, J. 1998. Ecology and Water Quality Dynamics of a Shallow Hypereutrophic Lake Dominated by Cyanobacteria (*Aphanizomenon flos-aquae*). Ph.D. dissertation, University of North Carolina, Chapel Hill, NC.

Kann, J. and V.H. Smith. 1999. Estimating the probability of exceeding elevated pH values

critical to fish populations in a hypereutrophic lake. *Can. J. Fish. Aquat. Sci.* 56:2262-2270.
Kann, J. and W.W. Walker. 1999. Nutrient and Hydrologic Loading to Upper Klamath Lake, Oregon, 1991-1998. Report submitted to Klamath Tribes Natural Resources Department and U.S. Bureau of Reclamation.
Lang, T., G. Peters, R. Hoffmann and E. Meyer. 1987. Experimental investigations on the toxicity of ammonia: effects on ventilation frequency, growth, epidermal mucous cells, and gill structure of rainbow trout *Salmo gairdneri*. *Dis. Aquat. Org.* 3:159-165.
Laurent, P. 1989. Gill structure and function: fish. pp. 69-120 in: S.C. Wood (ed.). Comparative Pulmonary Physiology. Marcel Dekker, Inc., New York, NY.
Lewis, P.A., D.J. Klemm, J.M. Lazorchak, T.J. Norberg-King, W.H. Peltier and M.A. Heber (eds.). 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third Edition. EPA-600-4-91-002, U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH.
Little, E.E., R.D. Archeski, B.A. Flerov and V.I. Kozlovskaya. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. *Arch. Environ. Contam. Toxicol.* 19:380-385.
Maetz, J. 1973. Na^+/NH_4^+, Na^+/H^+ exchanges and NH_3 movement across the gill of *Carassius auratus*. *J. Exp. Biol.* 58:255-275.
Martin, B.A. 1997. Effects of Ambient Water Quality on the Endangered Lost River Sucker *Deltistes luxatus* in Upper Klamath Lake, Oregon. M.S. Thesis, Humboldt State University, Arcata, CA.
Martin, B.A. and M.K. Saiki. 1999. Effects of ambient water quality on the endangered Lost River sucker in Upper Klamath Lake, Oregon. *Trans. Am. Fish. Soc.* 128:953-961.
McDonald, D.G., W.J. McFarlane and C.L. Milligan. 1998. Anaerobic capacity and swim performance of juvenile salmonids. *Can. J. Fish. Aquat. Sci.* 55:1198-1207.
McGeer, J.C., P.A. Wright, C.M. Wood, M.P. Wilkie, C.F. Mazur and G.K. Iwama. 1994. Nitrogen excretion in four species of fish from an alkaline lake. *Trans. Am. Fish. Soc.* 123:824-829.
Monda, D. and M.K. Saiki. 1993. Chapter 8 -- Tolerance of juvenile Lost River and shortnose suckers to high pH, ammonia concentration, and temperature, and to low dissolved oxygen concentration. pp. 279-341 in: Environmental Research in the Klamath Basin, Oregon: 1992 Annual Report. U.S. Department of the Interior, Bureau of Reclamation, Denver, CO.
Monda, D. and M.K. Saiki. 1994. Tolerance of Larval Lost River Sucker to High pH, Ammonia Concentration, and Temperature, and to Low Dissolved Oxygen Concentration. National Biological Survey, National Fisheries Contaminant Research Center, Field Research Station-Dixon, Dixon, CA.
Morgan, M. and P.W.A. Tovell. 1973. The structure of the gill of the trout, *Salmo gairdneri* (Richardson). *Z. Zellforsch. Mikrosk. Anat.* 142:147-162.
Mueller, M.E., D.A. Sanchez, H.L. Bergman, D.G. McDonald, R.G. Rhem and C.M. Wood. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. *Can. J. Fish. Aquat. Sci.* 48:2016-2027.
Neter, J., W. Wasserman and M.H. Kutner. 1990. Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs. Third Edition. Irwin, Homewood, IL.
Newstead, J.D. 1967. Fine structure of the respiratory lamellae of teleostean gills. *Z. Zellforsch. Mikrosk. Anat.* 79:96-428.

Perkins, D.L., J. Kann and G.G. Scoppettone. 199_. The role of poor water quality and fish kills in the decline of endangered Lost River and shortnose suckers in Upper Klamath Lake. Draft manuscript.

Post, G. 1987. Textbook of Fish Health. Revised and Expanded Edition. T.F.H. Publications, Inc., Neptune City, NJ.

Powell, M.D., D.J. Speare and G.M. Wright. 1994. Comparative ultrastructural morphology of lamellar epithelial, chloride and mucous cell glycocalyx of the rainbow trout (*Oncorhynchus mykiss*) gill. *J. Fish Biol.* 44:725-730.

Russo, R.C. 1985. Ammonia, nitrite, and nitrate. pp. 455-471 in: G.M. Rand and S.R. Petrocelli (eds.). Fundamentals of Aquatic Toxicology: Methods and Applications. Hemisphere Publishing Corporation, New York, NY.

Saiki, M.K., D.P. Monda and B.L. Bellerud. 1999. Lethal levels of selected water quality variables to larval and juvenile Lost River and shortnose suckers. *Environ. Pollut.* 105:37-44.

Smart, G. 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* 8:471-475.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Company, San Francisco, CA.

Tietge, J.E., R.D. Johnson and H.L. Bergman. 1988. Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long-term exposure to acid and aluminum. *Can. J. Fish. Aquat. Sci.* 45:1643-1648.

Ultsch, G.R. and G. Gros. 1979. Mucus as a diffusion barrier to oxygen: possible role in O_2 uptake at low pH in carp (*Cyprinus carpio*) gills. *Comp. Biochem. Physiol.* 62A:685-689.

USFWS (U.S. Fish and Wildlife Service). 1988. Endangered and threatened wildlife and plants -- shortnose and Lost River suckers, Houghton's goldenrod and Pitcher's thistle: final rules. Federal Register 53:27130-27134.

USFWS (U.S. Fish and Wildlife Service). 1993. Recovery Plan: Lost River Sucker (*Deltistes luxatus*) and Shortnose Sucker (*Chasmistes brevirostris*). U.S. Department of the Interior, Fish and Wildlife Service, Portland, OR.

Verdouw, H., C.J.A. van Echteld and E.M.J. Dekkers. 1978. Ammonia determination based on indophenol formation with sodium salycilate. *Water Res.* 12:399-402.

WEST, Inc. and D.D. Gulley. 1994. TOXSTAT® 3.4. Western EcoSystems Technology, Inc., Cheyenne, WY.

Weibel, E.R. 1979. Practical Methods for Biological Morphometry. Volumes 1 and 2. Academic Press Inc., London, UK.

Wilson, R.W., P.M. Wright, S. Munger and C.M. Wood. 1994. Ammonia excretion in freshwater rainbow trout (*Oncorhynchus mykiss*) and the importance of gill boundary layer acidification: lack of evidence for Na^+/NH_4^+ exchange. *J. Exp. Biol.* 191:37-58.

Wood, C.M., S.F. Perry, P.A. Wright, H.L. Bergman and D.J. Randall. 1989. Ammonia and urea dynamics in the Lake Magadi tilapia, a ureotelic teleost fish adapted to an extremely alkaline environment. *Respir. Physiol.* 77:1-20.

Table 1. Water chemistry in toxicity tests. Means and standard deviations (s.d.) were calculated from daily parameters measured in all exposure aquaria.

Toxicity test	Mean temperature (C) (±s.d.)	Mean pH (±s.d.)	Mean hardness (mg/L as $CaCO_3$) (±s.d.)	Mean alkalinity (mg/L as $CaCO_3$) (±s.d.)	Mean conduc ivity (µS/cm (±s.d.)
#1 -- Low dissolved oxygen concentrations (with access to water surface)	22.4 (±0.41) n = 14	8.09 (±0.079) n = 14	50.1 (±1.60) n = 14	50.9 (±2.07) n = 14	133 (±6.4) n = 14
#2 -- Low dissolved oxygen concentrations (without access to water surface)	22.0 (±0.33) n = 14	7.99 (±0.069) n = 14	51.1 (±3.11) n = 14	52.9 (±4.90) n = 14	121 (±6.4) n = 14
#3 -- Elevated pH	21.6 (±0.27) n = 30	varied among treatments	53.9 (±3.84) n = 30	53-107 (covaried with pH)[a]	135-26 (covarie with pH
#4 -- Elevated ammonia concentrations at pH 9.5	22.3 (±0.22) n = 31	9.47 (±0.093) n = 31	51.1 (±6.48) n = 31	71.1 (±9.09) n = 30	162 (±17.8) n = 31
#5 -- Sublethal ammonia concentrations at pH 9.5, followed by nonlethal dissolved oxygen concentrations	22.1 (±0.33) n = 30	7.5-9.5 (differed between the two phases)	49.2 (±4.81) n = 30	43-65 (differed between the two phases)[a]	111-17 (differe betwee the tw phases

[a] Alkalini ty and conductivity increased as pH increased because KOH was added to the exposure waters to in
[b] Ammonia concentrations varied considerably and sometimes were high because water samples for ammonia immediately after the fish were fed.

Table 2. Results of Toxicity Test #1 -- late-juvenile Lost River suckers exposed to low dissolved oxygen (D access to the water surface) for 14 days. * = significantly different from control (P < 0.05); ‡ = tr the statistical analysis because of significantly decreased survival; --- = not measured.

Nominal DO conc. (mg/L)	Mean DO conc. (mg/L) (±s.d.)	Mean survival (%) (±s.d.) n=4/trtmt	Mean frequency of scoliosis[a] (%) (±s.d.) n=4/trtmt	Mean final length[b] (mm) (±s.d.) n=4/trtmt	Mean final weight[b] (g) (±s.d.) n=4/trtmt	Mean whole-body ion content (mg/g dw) (±s.d.) n=4/trtmt				Median time to exhaustio at critica swimmin speed[c] ((range)
						Ca	K	Na	Cl	
1	1.54 (±0.38)	33.3 * (±16.2)	0.0 ‡ (±0.00)	70.2 ‡ (±1.30)	2.91 ‡ (±0.048)	21.4 ‡ (±2.85)	12.9 ‡ (±1.12)	5.06 ‡ (±0.206)	3.04 ‡ (±0.112)	---
2	1.99 (±0.34)	95.0 (±6.27)	1.7 (±3.34)	72.5 (±2.70)	3.60 (±0.289)	19.7 (±3.74)	12.1 (±0.48)	5.09 (±0.186)	2.84 (±0.192)	41 (29-159 n = 4
3	2.99 (±0.31)	96.5 (±4.04)	3.3 (±3.85)	73.5 (±1.71)	3.77 (±0.246)	14.3 (±2.80)	13.8 (±0.84)	5.25 (±0.584)	3.06 (±0.462)	129 (38->60 n = 4
4	3.85 (±0.28)	100.0 (±0.00)	10.0 (±8.61)	72.6 (±1.70)	3.75 (±0.309)	17.7 (±5.18)	12.8 (±0.92)	5.11 (±0.260)	2.89 (±0.236)	78 (42->60 n = 3
5	4.71 (±0.34)	100.0 (±0.00)	10.0 (±8.61)	72.7 (±1.08)	3.71 (±0.221)	18.7 (±2.65)	12.4 (±1.19)	5.26 (±0.572)	3.00 (±0.198)	84 (35-310 n = 3
7 (control)	6.29 (±0.37)	100.0 (±0.00)	8.3 (±10.00)	73.7 (±1.53)	3.80 (±0.187)	19.7 (±2.46)	12.7 (±0.70)	5.24 (±0.464)	2.76 (±0.484)	>34 (9-106) n = 4

[a] Frequency of scoliosis in all fish (dead and alive).
[b] Fish with scoliosis were not included in these treatment means and the statistical analyses.
[c] Median time to exhaustion in each sample of ≤5 fish that were swum per replicate tank was used to calculat replicates per treatment. One-tailed Wilcoxon's Rank Sum Test was used to test for significance among trea

Table 3. Results of Toxicity Test #2 -- late-juvenile Lost River suckers exposed to low dissolved oxygen (I (without access to the water surface) for 14 days. * = significantly different from control (P < 0.05 included in the statistical analysis because of significantly decreased survival; --- = not measured

Nominal DO conc. (mg/L)	Mean DO conc. (mg/L) (±s.d.)	Mean survival (%) (±s.d.) n=4/trtmt	Mean frequency of scoliosis[a] (%) (±s.d.) n=4/trtmt	Mean final length[b] (mm) (±s.d.) n=4/trtmt	Mean final weight[b] (g) (±s.d.) n=4/trtmt	Mean whole-body ion content (mg/g dw) (±s.d.) n=4/trtmt				Mean swimmin speed a exhaustic (bl/s)[c] (±s.d.) n=4/trtn
						Ca	K	Na	Cl	
1	1.44 (±0.20)	41.7 * (±17.5)	0.0 ‡ (±0.00)	71.2 ‡ (±5.80)	3.19 ‡ (±0.755)	---	12.2 ‡ (±0.36)	4.76 ‡ (±0.175)	5.01 ‡ (±0.373)	---
2	2.12 (±0.12)	98.3 (±3.33)	3.3 (±3.85)	73.4 (±1.97)	3.55 (±0.383)	---	12.4 (±0.50)	4.40 (±0.136)	4.68 (±0.254)	4.43 (±0.587
3	3.05 (±0.12)	98.3 (±3.33)	6.7 (±5.44)	73.3 (±2.27)	3.61 (±0.396)	---	12.8 (±0.55)	4.48 (±0.221)	4.92 (±0.175)	5.04 (±0.420
4	4.05 (±0.14)	98.3 (±3.33)	3.3 (±3.85)	72.4 (±2.24)	3.49 (±0.361)	---	13.0 (±0.23)	4.65 (±0.089)	4.91 (±0.113)	4.95 (±0.478
5	4.90 (±0.20)	100.0 (±0.00)	5.0 (±6.38)	73.6 (±2.05)	3.69 (±0.266)	---	12.9 (±0.45)	4.60 (±0.234)	4.98 (±0.331)	---
7 (control)	6.00 (±0.24)	100.0 (±0.00)	1.7 (±3.34)	71.2 (±0.86)	3.29 (±0.101)	---	12.8 (±0.33)	4.63 (±0.085)	5.06 (±0.359)	4.81 (±0.192

[a] Frequency of scoliosis in all fish (dead and alive).
[b] Fish with scoliosis were not included in these treatment means and the statistical analyses.
[c] bl/s = body lengths/second.

Table 4. Water chemistry in Toxicity Test #3 -- larval Lost River suckers exposed to elevated pH for 30 da
deviations (s.d.) were calculated from daily means of water quality parameters measured in all expo

Nominal pH	Mean temperature (C) (±s.d.)	Mean pH (±s.d.)	Mean hardness (mg/L as $CaCO_3$) (±s.d.)	Mean alkalinity (mg/L as $CaCO_3$) (±s.d.)	Mean conductivity (µS/cm) (±s.d.)
8.0 (control)	21.6 (±0.26) n = 30	7.96 (±0.180) n = 30	53.5 (±4.74) n = 30	52.7 (±4.47) n = 30	135 (±25.0) n = 30
9.0	21.6 (±0.27) n = 30	8.98 (±0.073) n = 30	52.2 (±5.96) n = 30	59.6 (±6.44) n = 30	171 (±54.8) n = 30
9.5	21.8 (±0.32) n = 30	9.46 (±0.084) n = 30	53.9 (±5.68) n = 30	72.1 (±10.76) n = 30	166 (±20.3) n = 30
10.0	21.3 (±0.43) n = 30	9.98 (±0.112) n = 30	56.1 (±6.31) n = 30	107.3 (±23.57) n = 30	261 (±53.1) n = 30

Table 5. Results of Toxicity Test #3 -- larval Lost River suckers exposed to elevated pH for 30 days. * = s from control ($P < 0.05$).

Nominal pH	Mean pH (±s.d.) n=4/trtmt	Mean survival (%) (±s.d.) n=4/trtmt	Mean frequency of scoliosis[a] (%) (±s.d.) n=4/trtmt	Mean final length[b] (mm) (±s.d.) n=4/trtmt	Mean final weight[b] (g) (±s.d.) n=4/trtmt	Mean whole-body ion content (mg/g dw) (±s.d.) n=4/trtmt				Median time to exhaustic at critica swimmin speed[c] ((range)
						Ca	K	Na	Cl	
8.0 (control)	7.96 (±0.18)	98.9 (±2.18)	1.0 (±2.08)	32.4 (±0.42)	0.238 (±0.010)	26.0 (±1.07)	17.6 (±0.40)	6.00 (±0.111)	6.56 (±1.454)	22 (0->1,20 n = 4
9.0	8.98 (±0.07)	96.0 (±4.63)	6.1 (±7.93)	33.9 (±1.66)	0.280 (±0.044)	25.2 (±1.18)	17.8 (±0.60)	5.71 (±0.448)	7.26 (±0.205)	57 (40->1,2(n = 3
9.5	9.46 (±0.08)	97.7 (±2.64)	1.1 (±2.18)	34.2 (±1.69)	0.286 (±0.050)	25.6 (±2.66)	17.9 (±0.63)	5.73 (±0.418)	6.13 (±1.593)	54 (0-325) n = 3
10.0	9.98 (±0.11)	91.8 (±10.22)	5.5 (±6.40)	32.7 (±0.44)	0.237 (±0.072)	24.9 (±0.96)	18.0 (±0.36)	5.19 * (±0.317)	7.34 (±0.174)	2 (0-5) n = 4

[a] Frequency of scoliosis in all fish (dead and alive).
[b] Fish with scoliosis were not included in these treatment means and the statistical analyses.
[c] Median time to exhaustion in each sample of ≤5 fish that were swum per replicate tank (at an average speed was used to calculate the median of the three or four replicates per treatment. One-tailed Wilcoxon's Rank test for significance among treatments.

Table 6. Results of Toxicity Test #4 -- larval Lost River suckers exposed to elevated ammonia concentratio NH_3-N = unionized ammonia nitrogen; * = significantly different from control ($P < 0.05$); ‡ = tre the statistical analysis because of significantly decreased survival; --- = not measured.

Nominal NH_3-N conc. (mg/L)	Mean NH_3-N conc. (mg/L) (±s.d.)	Mean survival (%) (±s.d.) n=4/trtmt	Mean frequency of scoliosis[a] (%) (±s.d.) n=4/trtmt	Mean final length[b] (mm) (±s.d.)	Mean final weight[b] (g) (±s.d.)	Mean whole-body ion content (mg/g dw) (±s.d.)				Median time to exhaustio at critica swimmin speed[c] ((range)
						Ca	K	Na	Cl	
0 (control)	0.01 (±0.01) n = 31	92.0 (±6.98)	9.6 (±7.29)	37.2 (±0.39) n = 4	0.359 (±0.009) n = 4	25.0 (±3.15) n = 4	16.5 (±0.35) n = 4	5.39 (±0.082) n = 4	6.60 (±0.447) n = 4	66 (58-142 n = 4
0.075	0.11 (±0.06) n = 31	93.4 (±9.96)	6.7 (±9.94)	36.6 (±1.11) n = 4	0.338 (±0.027) n = 4	27.5 (±5.37) n = 4	15.4 (±1.09) n = 4	5.26 (±0.505) n = 4	6.53 (±0.466) n = 4	>271 (59->1,20 n = 4
0.15	0.20 (±0.04) n = 31	98.5 (±2.94)	0.0 (±0.00)	36.5 (±0.98) n = 4	0.331 (±0.033) n = 4	27.2 (±3.88) n = 4	15.9 (±0.44) n = 4	5.22 (±0.336) n = 4	6.34 (±0.262) n = 4	49 (23-156 n = 4
0.3	0.37 (±0.06) n = 31	71.4 (±47.74)	0.0 (±0.00)	37.1 (±2.92) n = 3	0.353 (±0.064) n = 3	26.9 (±1.30) n = 3	15.8 (±0.51) n = 3	5.15 (±0.479) n = 3	6.40 (±0.539) n = 3	61 (25-150 n = 3
0.6	0.69 (±0.10) n = 30	1.4 * (±2.78)	0.0 ‡ (±0.00)	26.0 ‡ (±0.00) n = 1	0.105 ‡ (±0.000) n = 1	---	---	---	---	---
1.2	1.16 (±0.18) n = 15	0.0 * (±0.00)	0.0 ‡ (±0.00)	---	---	---	---	---	---	---

[a] Frequency of scoliosis in all fish (dead and alive).
[b] Fish with scoliosis were not included in these treatment means and the statistical analyses.
[c] Median time to exhaustion in each sample of ≤5 fish that were swum per replicate tank (at an average speed was used to calculate the median of the three or four replicates per treatment. One-tailed Wilcoxon's Rank test for significance among treatments.

Table 7. Water chemistry during elevated-ammonia phase (days 1-14) of Toxicity Test #5 -- early juvenile exposed to sublethal ammonia concentrations at pH 9.5 for 14 days, followed by exposure to subl concentrations for 14 days. Means and standard deviations (s.d.) were calculated from daily mea parameters measured in all exposure aquaria.

Nominal unionized ammonia conc. (mg NH_3-N/L)	Mean temperature (C) (±s.d.)	Mean pH (±s.d.)	Mean hardness (mg/L as $CaCO_3$) (±s.d.)	Mean alkalinity (mg/L as $CaCO_3$) (±s.d.)	Mean conductivity (µS/cm) (±s.d.)
0	21.8 (±0.27) n = 15	9.50 (±0.054) n = 15	50.6 (±4.08) n = 15	62.3 (±8.19) n = 15	171 (±61.8) n = 15
0.15	22.0 (±0.12) n = 15	9.49 (±0.056) n = 15	51.8 (±4.38) n = 15	65.2 (±9.71) n = 15	165 (±35.1) n = 15
0.3	21.9 (±0.17) n = 15	9.48 (±0.056) n = 15	49.6 (±4.61) n = 15	61.8 (±8.96) n = 15	163 (±25.7) n = 15

Table 8. Water chemistry during low-dissolved-oxygen phase (days 16-29) of Toxicity Test #5 -- early juv exposed to sublethal ammonia concentrations at pH 9.5 for 14 days, followed by exposure to subl concentrations for 14 days. Means and standard deviations (s.d.) were calculated from daily mea parameters measured in all exposure aquaria.

Nominal unionized ammonia conc. (mg NH_3-N/L)	Nominal dissolved oxygen conc. (mg/L)	Mean temperature (C) (±s.d.)	Mean pH (±s.d.)	Mean hardness (mg/L as $CaCO_3$) (±s.d.)	Mean alkalinity (mg/L as $CaCO_3$) (±s.d.)	Mean conductivity (µS/cm) (±s.d.)
0 (control)	7 (control)	22.4 (±0.51) n = 15	7.95 (±0.131) n = 15	49.8 (±8.89) n = 28	49.8 (±7.87) n = 29	139 (±15.0) n = 15
0	3	22.2 (±0.38) n = 15	7.99 (±0.116) n = 15	49.8 (±8.89) n = 28	49.8 (±7.87) n = 29	118 (±4.0) n = 15
0	2	22.3 (±0.40) n = 15	8.03 (±0.082) n = 15	49.8 (±8.89) n = 28	49.8 (±7.87) n = 29	112 (±4.7) n = 15
0.15	7	22.4 (±0.52) n = 15	7.95 (±0.138) n = 15	46.1 (±6.50) n = 28	43.5 (±4.27) n = 29	139 (±14.8) n = 15
0.15	3	22.3 (±0.37) n = 15	8.02 (±0.085) n = 15	46.1 (±6.50) n = 28	43.5 (±4.27) n = 29	118 (±3.7) n = 15
0.15	2	22.3 (±0.38) n = 15	8.04 (±0.094) n = 15	46.1 (±6.50) n = 28	43.5 (±4.27) n = 29	111 (±4.4) n = 15

Table 8 (continued).

Nominal unionized ammonia conc. (mg NH_3-N/L)	Nominal dissolved oxygen conc. (mg/L)	Mean temperature (C) (±s.d.)	Mean pH (±s.d.)	Mean hardness (mg/L as $CaCO_3$) (±s.d.)	Mean alkalinity (mg/L as $CaCO_3$) (±s.d.)	Mean conductivity (µS/cm) (±s.d.)
0.3	7	22.3 (±0.54) n = 15	7.98 (±0.115) n = 15	47.1 (±4.28) n = 28	46.5 (±5.04) n = 29	138 (±14.5) n = 15
0.3	3	22.3 (±0.36) n = 15	8.00 (±0.104) n = 15	47.1 (±4.28) n = 28	46.5 (±5.04) n = 29	119 (±4.9) n = 15
0.3	2	22.2 (±0.40) n = 15	8.04 (±0.092) n = 15	47.1 (±4.28) n = 28	46.5 (±5.04) n = 29	112 (±4.9) n = 15

Table 9. Results of Toxicity Test #5 -- early juvenile Lost River suckers exposed to sublethal ammonia con 14 days, followed by exposure to sublethal dissolved oxygen concentrations for 14 days. NH_3-N nitrogen; --- = not measured.

Nominal NH_3-N conc. (mg/L)	Nominal DO conc. (mg/L)	Mean survival during sublethal DO phase (%) (±s.d.) n=4/trtmt	Mean freq-uency of scoliosis[a] (%) (±s.d.) n=4/trtmt	Mean final length[b] (mm) (±s.d.) n=4/trtmt	Mean final weight[b] (g) (±s.d. n=4/trtmt	Mean whole-body ion content (mg/g dw) (±s.d.) n = 4/trtmt				Median time to exhaustio at critica swimmin speed[c] ((range) n=4/trtn
						Ca	K	Na	Cl	
0 (control)	7 (control)	100.0 (±0.00)	0.0 (±0.00)	41.9 (±1.12)	0.515 (±0.016)	35.4 (±1.41)	18.8 (±0.29)	5.83 (±0.078)	7.01 (±0.227)	135 (49->60
0	3	100.0 (±0.00)	0.0 (±0.00)	43.4 (±0.54)	0.508 (±0.018)	35.4 (±0.71)	18.8 (±0.52)	5.74 (±0.158)	7.21 (±0.371)	76 (68->60
0	2	89.9 (±16.98)	0.0 (±0.00)	41.6 (±0.31)	0.516 (±0.020)	35.3 (±0.75)	18.8 (±0.38)	5.85 (±0.104)	7.34 (±0.280)	76 (49-130
0.15	7	100.0 (±0.00)	0.0 (±0.00)	43.3 (±0.79)	0.532 (±0.034)	35.0 (±0.61)	18.7 (±0.45)	5.67 (±0.087)	7.02 (±0.017)	70 (44->60
0.15	3	100.0 (±0.00)	0.0 (±0.00)	41.2 (±0.91)	0.515 (±0.027)	34.9 (±1.23)	19.0 (±0.22)	5.87 (±0.091)	7.43 (±0.251)	183 (56->60
0.15	2	94.9 (±0.24)	0.0 (±0.00)	43.1 (±0.30)	0.523 (±0.011)	34.0 (±0.99)	18.6 (±0.28)	5.85 (±0.021)	7.45 (±0.138)	57 (40-207

Table 9 (continued).

Nominal NH_3-N conc. (mg/L)	Nominal DO conc. (mg/L)	Mean survival during sublethal DO phase (%) (±s.d.) n=4/trtmt	Mean freq-uency of scoliosis[a] (%) (±s.d.) n=4/trtmt	Mean final length (mm) (±s.d.) n=4/trtmt	Mean final weight (g) (±s.d.) n=4/trtmt	Mean whole-body ion content (mg/g dw) (±s.d.) n=4/trtmt				Median time to exhaustio at critica swimmin speed[b] ((range) n=4/trtm
						Ca	K	Na	Cl	
0.3	7	100.0 (±0.00)	0.0 (±0.00)	41.6 (±1.14)	0.532 (±0.020)	35.6 (±1.36)	18.5 (±0.47)	5.74 (±0.148)	7.10 (±0.129)	90 (66-115
0.3	3	100.0 (±0.00)	0.0 (±0.00)	43.4 (±1.55)	0.526 (±0.040)	35.6 (±1.57)	19.1 (±0.26)	5.86 (±0.387)	7.23 (±0.643)	95 (81-239
0.3	2	89.3 (±13.41)	0.0 (±0.00)	42.5 (±0.61)	0.565 (±0.025)	34.4 (±0.78)	18.4 (±0.48)	5.90 (±0.132)	7.51 (±0.179)	79 (61->60

[a] Frequency of scoliosis in all fish (dead and alive).
[b] Median time to exhaustion in each sample of ≤5 fish that were swum per replicate tank (at an average speed was used to calculate the median of the three or four replicates per treatment. One-tailed Wilcoxon's Rank test for significance among treatments.

Table 10. Water-quality conditions during acclimation periods prior to temperature-dependent swimming-per juvenile Lost River suckers.

Parameter	Early juveniles					Late ju		
	7 C	10 C	15 C	20 C	25 C	7 C	10 C	15
Temperature (C)	7.5	10.5	14.9	20.4	25.0	7.7	9.9	15
pH	7.7	7.8	7.8	7.6	7.5	7.8	7.7	7
Alkalinity (mg/L as $CaCO_3$)	200	188	183	183	190	182	183	202
Hardness (mg/L as $CaCO_3$)	184	185	183	190	190	197	179	189
Conductivity (µS/cm)	449	420	449	459	455	455	437	477
Dissolved oxygen (mg/L)	7.3	7.7	6.3	5.8	6.0	6.4	7.0	7

Table 11. Water-quality conditions, fish sizes, and results of temperature-dependent swimming-performance River suckers. CSS = critical swimming speed (in cm/sec) ; CSS_{BL} = body-length-normalized criti body lengths/sec).

Parameter		Early juveniles 7 C	10 C	15 C	20 C	25 C	Late juveniles 7 C	10 C	15 C	20
Temperature		7.3	10.5	14.9	20.2	25.0	7.4	10.2	15.0	20
(C)		7.2-7.5	10.5-10.5	14.8-15.0	20.2-20.2	25.0-25.0	6.9-7.6	9.9-10.3	15.0-15.2	20.2-
pH		7.7	7.8	7.9	7.6	7.6	7.8	7.7	7.8	7
		7.7-7.8	7.8-7.8	7.9-7.9	7.6-7.6	7.6-7.6	7.7-7.9	7.7-7.7	7.7-7.8	7.6
Alkalinity		199	178	171	186	191	195	177	193	189
(mg/L as $CaCO_3$)		197-200	178-178	168-175	186-186	191-191	187-199	177-177	193-193	189-1
Hardness		192	185	174	203	189	193	188	171	198
(mg/L as $CaCO_3$)		191-195	185-185	164-188	203-203	189-189	190-196	188-188	171-171	198-1
Conductivity		460	409	413	462	454	454	438	393	459
(µS/cm)		459-460	409-409	389-449	462-462	454-454	447-458	438-438	393-393	459-4
Dissolved		6.9	7.4	6.7	6.1	6.3	6.6	7.0	6.7	6
oxygen (mg/L)		6.8-7.0	7.3-7.4	6.4-7.0	6.1-6.1	5.9-6.6	6.4-6.9	6.9-7.3	6.6-6.8	6.5
Fish length		47.0	48.1	49.2	49.4	49.6	74.6	75.4	73.8	76
(mm)		39-54	42-58	40-57	39-64	43-56	67-88	64-89	60-96	64
Fish mass		0.77	0.82	0.81	0.88	0.88	3.64	3.73	3.58	3
(g)		0.40-1.42	0.52-1.81	0.47-1.32	0.40-1.97	0.50-1.55	2.51-5.72	2.22-6.15	2.10-6.84	2.02-
CSS	ave.	21.0	18.3	22.1	29.0	25.6	17.3	25.6	27.3	29
(cm/s)	s.d.	2.5	2.2	3.5	4.3	5.8	3.0	3.8	6.9	1
	median	20.7	18.6	22.3	31.3	24.0	16.2	26.4	28.0	28
	25%-75%	19.7-21.7	16.7-19.9	20.3-24.3	26.9-32.1	20.9-31.4	15.1-19.2	22.2-27.9	21.7-32.0	27.6-
	10%-90%	18.4-25.2	14.9-21.1	16.5-25.7	22.7-32.3	18.5-32.7	14.2-21.4	19.9-30.7	19.2-36.1	26.5-
	min-max	15.6-27.2	13.3-22.0	15.7-31.3	14.4-32.4	14.4-34.4	13.2-25.7	18.7-32.4	14.2-45.2	26.2-
CSS_{BL}	ave.	4.48	3.80	4.50	5.88	5.15	2.32	3.41	3.73	3
(bl/s)	s.d.	0.53	0.41	0.70	0.87	1.11	0.34	0.53	0.93	0
	median	4.41	3.87	4.50	6.20	4.79	2.34	3.32	4.00	3
	25%-75%	4.08-4.74	3.52-4.13	4.08-4.86	5.26-6.42	4.40-6.16	2.04-2.49	2.93-3.76	3.02-4.20	3.58-
	10%-90%	3.86-5.24	3.11-4.28	3.49-5.25	4.67-6.98	3.86-6.44	1.87-2.80	2.76-4.16	2.24-4.96	3.32-
	min-max	3.39-5.78	2.96-4.50	3.27-6.51	3.69-7.20	2.83-7.53	1.81-3.26	2.64-4.57	1.95-5.51	3.27-
	n	24	25	25	25	24	25	25	25	2

Table 12. Median lethal concentrations (LC50s) and chronic-effects concentrations in the published literature study, for larval and juvenile Lost River suckers (LRS) and shortnose suckers (SNS) exposed to l (DO) concentrations.

Species & life stage	Mean body weight (g) (range)	LC50 (mg DO/L) (95% confidence interval) 24-h	48-h	96-h	30-d	ChV[a] (mg/L) (HOEC, NOEC
LRS larvae	---[c]	2.01 (1.90-2.13)	2.10 (2.07-2.13)	2.10 (2.07-2.13)	---	---
SNS larvae	---	1.92 (1.89-1.96)	2.04 (1.90-2.18)	2.09 (1.90-2.29)	---	---
LRS juveniles	--- (0.39-0.86)	1.58 (1.35-1.86)	1.58 (1.35-1.86)	1.62 (1.41-1.86)	---	---
SNS juveniles	--- (0.39-1.15)	1.14 (0.84-1.55)	1.34 (1.15-1.55)	1.34 (1.15-1.55)	---	---
LRS late-juveniles	3.63 (2.22-4.96)	1.55 (1.47-1.64)	1.58 (1.52-1.64)	1.57 (1.52-1.64)	1.64 (1.59-1.69)	1.75 (1.54, 1.99)
LRS late-juveniles	3.65 (1.71-7.27)	<1.21[d]	<1.16[d]	1.27 (1.18-1.39)	1.51 (1.43-1.61)	1.75 (1.44, 2.12)

[a] ChV = chronic value = geometric mean of HOEC and NOEC.
[b] HOEC = highest observed effects concentration; NOEC = no observed effects concentration.
[c] --- = not determined.
[d] LC50 could not be calculated because mortality was <50% in lowest DO concentration tested.

Table 13. Median lethal concentrations (LC50s) and chronic-effects concentrations in the published literature study, for larval and juvenile Lost River suckers (LRS) and shortnose suckers (SNS) exposed to e

Species & life stage	Mean body weight (g) (range)	LC50 (pH units) (95% confidence interval) 24-h	48-h	96-h	30-d	ChV[a] (pH units (NOEC, LOEC
LRS larvae	0.041 (0.031-0.054)	>10.0[c]	>10.0[c]	>10.0[c]	>10.0[c]	9.75 (9.5, 10.0)
LRS larvae	---[d]	10.42 (10.38-10.47)	10.39 (10.32-10.46)	10.35 (10.26-10.45)	---	---
SNS larvae	---	10.38 (10.31-10.46)	10.38 (10.31-10.46)	10.38 (10.31-10.46)	---	---
LRS juveniles	--- (0.28-0.49)	10.66 (10.59-10.74)	10.62 (10.54-10.71)	10.30 (9.94-10.67)	---	---
SNS juveniles	--- (1.01-1.11)	10.69 (10.61-10.77)	10.66 (10.61-10.72)	10.39 (10.22-10.56)	---	---

[a] ChV = chronic value = geometric mean of NOEC and LOEC.
[b] NOEC = no observed effects concentration; LOEC = lowest observed effects concentration.
[c] LC50 could not be calculated because the highest pH tested did not cause 50% mortality.
[d] --- = not determined.

Table 14. Median lethal concentrations (LC50s) and chronic-effects concentrations in the published literatu study, for larval and juvenile Lost River suckers (LRS) and shortnose suckers (SNS) exposed to e ammonia concentrations.

Species & life stage	Mean body weight (g) (range)	LC50 (mg NH_3-N/L) (95% confidence interval) 24-h	48-h	96-h	30-d	ChV[a] (mg NH_3-N/L (NOEC, LOEC
LRS larvae	0.059 (0.033-0.109	>1.16[c]	0.79 (0.78-0.79)	0.91 (0.88-0.93)	0.45 (0.42-0.48)	0.51 (0.37, 0.69)
LRS larvae	---[d]	0.56 (0.52-0.61)	0.51 (0.47-0.55)	0.48 (0.44-0.52)	---	---
SNS larvae	---	1.29 (0.83-2.00)	1.24 (0.82-1.88)	1.06 (0.73-1.53)	---	---
LRS juveniles	--- (0.49-0.80)	1.02 (1.01-1.04)	0.92 (0.82-1.04)	0.78 (0.70-0.86)	---	---
SNS juveniles	--- (0.53-2.00)	0.51 (0.30-0.87)	0.48 (0.28-0.82)	0.53 (0.34-0.82)	---	---

[a] ChV = chronic value = geometric mean of NOEC and LOEC.
[b] NOEC = no observed effects concentration; LOEC = lowest observed effects concentration.
[c] LC50 could not be calculated because the highest ammonia concentration tested did not cause 50% mortalit
[d] --- = not determined.

Table 15. Slope, intercept, correlation coefficient (R), Type I error probability (P) and sample size (n) for regressions of critical swimming speed (CSS, in cm/s) or body-length-normalized critical swimming speed (CSS_{BL}, in body lengths/s) on body length (mm) for early- and late-juvenile Lost River suckers combined, at temperatures ranging from 7-25 C. Data are plotted in Figure 12.

Comparison	Temperature (C)	Slope	Intercept	R	P	n
CSS vs.	7	-0.0999	25.22	-0.440	0.002	49
body length	10	0.262	5.78	0.791	<0.001	50
	15	0.196	12.65	0.448	0.001	50
	20	0.0282	27.23	0.126	0.384	50
	25	0.252	13.00	0.655	<0.001	49
CSS_{BL} vs.	7	-0.0726	7.81	-0.899	<0.001	49
body length	10	-0.0154	4.56	-0.439	0.001	50
	15	-0.0322	6.10	-0.492	<0.001	50
	20	-0.0704	9.28	-0.846	<0.001	50
	25	-0.0310	6.65	-0.502	<0.001	49